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(54) Title: MATRIX METALLOPROTEASE

Met Asn Cys Gin Gin Leu Trp Leu Gly Phe Leu Leu Pro Met Thr Val Ser Gly Arg Val 20
Leu Gly Leu Ala Glu Val Ala Pro Val Tyr Leu Ser Gin Tyr Gly Tyr Leu Gin Lys 40
Pro Leu Glu Gly Ser Asn Asn Phe Lys Pro Glu Asp Ile Thr Glu Ala Leu Arg Ala Phe 60
Gln Glu Ala Ser Glu Leu Pro Val Ser Gly Gin Leu Asp Asp Ala Thr Arg Ala Arg Met 80
Arg Ctn Pro Arg Cys Glu Leu Glu Asp Pro Phe Asn Asn Ile Tyr Leu Lys Tyr Leu Leu 100
Leu Gly Arg Ile Arg Lys Ile Leu Thr Phe Arg Ile Leu Asn Leu Pro Ser Thr Leu 120
Pro Pro His Thr Ala Arg Ala Leu Arg Glu Ala Phe Glu Asp Thr Ser Asn Val Ala 140
Pro Leu Thr Phe Ctn Glu Val Glu Ala Gly Ala Asp Ile Arg Leu Ser Phe His Gly 160
Arg Glu Ser Ser Cys Ser Asn Thr Phe Asp Gly Pro Gly Arg Val Leu Ala His Ala 180
Asp Ile Pro Glu Leu Gly Ser Val His Phe Asp Glu Asp Glu Phe Thr Glu Gly Thr 200
Tyr Arg Gly Val Asn Leu Arg Ile Ile Ala Ala His Glu Val Glu His Ala Leu Gly Leu 220
Gly His Ser Arg Tyr Ser Ctn Ala Leu Met Ala Pro Val Val Tyr Glu Gly Tyr Arg Pro His 240
Phe Lys Leu His Pro Asp Asp Val Ala Gly Ile Ctn Ala Leu Tyr Gly Lys Ser Pro 260
Val Ile Arg Asp Glu Glu Glu Glu Thr Glu Leu Pro Thr Val Pro Pro Val Pro Thr 280
Glu Pro Ser Pro His Pro Asp Pro Cys Ser Ser Glu Leu Asp Ala Met Met Leu Gly Pro 300
Arg Gly Lys Thr Tyr Ala Phe Lys Val Val Val Val Ser Asp Ser Gly Pro 320
Gly Pro Leu Phe Arg Val Ser Ala Leu Thr Glu Gly Leu Pro Gly Asn Leu Asp Ala Ala 340
Val Tyr Ser Pro Arg Thr Ctn Ile His Phe Phe Lys Lys Asp Lys Val Thr Arg Tyr 360
Ile Asn Phe Lys Met Ser Pro Gly Phe Pro Lys Leu Asn Arg Val Glu Pro Asn Leu 380
Asp Ala Ala Leu Tyr Trp Pro Leu Asn Glu Lys Val Phe Leu Phe Lys Gly Ser Gly Tyr 400
Trp Gin Trp Asp Glu Leu Ala Arg Thr Asp Ser Ser Thr Pro Lys Pro Ile Lys Gly 420
Leu Phe Thr Gly Val Pro Asn Glu Pro Ser Ala Ala Met Ser Trp Glu Asp Gly Arg Val 440
Tyr Phe Phe Lys Gly Val Tyr Trp Arg Leu Asn Glu Leu Arg Val Glu Lys Gly 460
Tyr Pro Arg Asn Ile Ser His Asn Trp Met His Cys Arg Pro Arg Thr Ile Asp Thr Thr 480
Pro Ser Gly Gly Asn Thr Thr Pro Ser Gly Thr Ile Thr Leu Asp Thr Thr Leu Ser 500
Ala Thr Glu Thr Thr Phe Glu Tyr 500

(57) Abstract

This invention relates to a novel metalloprotease having a proteolytic activity, its partial peptide or a salt either of them, a DNA coding for the protein, a recombinant vector comprising the DNA, a transformant carrying the recombinant vector, a process for producing the protein, a pharmaceutical composition comprising the DNA, an antibody against the protein, a method for screening for a compound which activates or inhibits a proteolytic activity of the protein, a kit for screening for the compound, and a compound which activates or inhibits a proteolytic activity of the protein which is identified, by the screening method or the kit. The DNA coding for the protein of the present invention can be used as a therapeutic and prophylactic composition for a variety of diseases including diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis and herniated disk. Furthermore, the protein of the present invention is useful as a screening reagent for any compounds which activate or inhibit the function of the protein of the present invention. In addition, the antibody against the protein of the present invention specifically recognizes the protein of the present invention and can be used in the quantitative determination of the protein of the present invention in a test fluid.

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DESCRIPTION

MATRIX METALLOPROTEASE

5 Technical Field

The present invention relates to a novel matrix metalloprotease and a DNA coding for the metalloprotease.

10 Background Art

The extracellular matrix, which is a cell-supporting tissue composed mainly of collagens and proteoglycans, is profoundly involved in such events as cell development, inflammation, and tissue repair. The enzymes known to be associated with the degradation of extracellular matrix are (1) cathepsin D, etc. which belongs to the aspartic proteaseas, (2) cathepsin B, H, L, etc. which belong to the cysteine proteases, (3) plasmin, kallikrein, neutrophil elastase, tryptase, chymase, cathepsin G, etc. which belong to the serine proteases, and (4) metalloproteases are known. Also called matrix metalloproteases, these metalloproteases are known to be playing central roles in the degradation of extracellular matrix.

So far, in humans, 13 kinds of matrix metalloproteases such as collagenases, gelatinases stromelysins, and membrane-type matrix metalloproteases have been cloned and their nucleotide sequences and amino acid sequences have been reported (T. Takino et al., *Journal of Biological Chemistry*, **270**, 23013, 1995; J. M. P. Freije et al., *Journal of Biological Chemistry*, **269**, 16766, 1994; H. Wills et al., *European Journal of Biochemistry*, **231**, 602, 1995). All of these enzymes are zinc-dependent metalloproteases, in which the amino acid sequence of the zinc-binding domain: His-Glu-X-Gly-His-Ser-Leu-Gly-Leu-X-His-Ser is well

conserved, and their activities are inhibited by o-phenanthroline. Each of these enzymes is secreted in the latent form which is inactive with a propeptide at the N-terminus of the active enzyme. A conserved 5 domain consisting in the amino acid sequence of Met-Arg-Lys-Pro-Arg-Cys-Gly-Val-Pro-Asp is located near the C-terminal region of the propeptide. This domain is called "cysteine switch", and it controls a protease activity by coordinating the zinc atom at active center 10 with cysteine in the domain. While the latent enzymes are activated upon cleavage of the propeptide, three kinds of inhibitor proteins, named TIMP, have been reported and known to performing strict control of activity. It is also known that, *in vitro*, the latent 15 enzymes are activated by treatment with trypsin or aminophenyl-mercuric acetate.

Matrix metalloproteases are not only involved in the degradation of the extracellular matrix such as collagens, gelatins which are denatured collagens, 20 proteoglycans, fibronectins, laminins, elastins, etc. but also are in charge of activation of other matrix metalloproteases and inactivation of protease inhibitors such as α 1-protease inhibitor. Furthermore, it is known that these metalloproteases are associated 25 with solubilization of membrane proteins and cell surface proteins such as TNF, Fas ligand, IL-6 receptor, TNF-receptor, etc. and, as a result, modulate the death, differentiation, proliferation inhibition, proliferation and gene expression of cells.

It is known that physiologically matrix metalloprotease activities are elevated in ovulation, development and differentiation, osteogenesis, atretic uterus, vascularization, and other events. In morbid 30 states, those metalloprotease activities are elevated in rheumatoid arthritis, osteoarthritis, cancer (metastasis and invasion), periodontitis, corneal ulcer,

gastric ulcer, myocardiopathy, aneurysm, otosclerosis, epidermolysis bullosa, premature labor, and atherosclerosis, among other conditions. Conversely, it is known that the enzyme activities are suppressed 5 in fibroid lung, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis, etc. Recently the fourth membrane-type matrix metalloprotease has been cloned (X. S. Puente et al., Cancer Research, 56, 944, 1996), suggesting the likelihood that there exist still 10 other novel matrix metalloproteases.

Any novel matrix metalloproteases of human origin make it possible to develop new drugs which inhibit or stimulate the activity of the metalloprotease and are useful for the prevention and treatment of various 15 matrix metalloprotease-associated morbidities, such as rheumatoid arthritis, and osteoarthritis. Therefore, in the technological area to which the present invention pertains, there has been a standing need for isolating novel human matrix metalloproteases and 20 developing a method for high production of the proteins.

The inventors of the present invention have made extensive research for solving the above problems and succeeded in cloning cDNAs each having a novel 25 nucleotide sequence from human liver-derived and rat liver-derived cDNA libraries. They have found that the proteins encoded by these cDNAs are matrix metalloproteases. The present inventors have made further investigations based on these findings, and 30 accomplished the present invention.

Disclosure of Invention

The present invention provides:

(1) A protein comprising an amino acid sequence 35 represented by SEQ ID NO:1 or a substantially equivalent thereto, or a salt thereof,

- (2) The protein according to claim 1, which comprises an amino acid sequence represented by SEQ ID NO:2,
- (3) The protein according to (1), which is a metalloprotease,
- 5 (4) A partial peptide of the protein according to (1), or a salt thereof, which shows the activity of the protein according to (1),
- (5) An isolated DNA which contains a DNA comprising a nucleotide sequence coding for a protein according to 10 (1),
- (6) The DNA according to (5), which comprises a nucleotide sequence represented by SEQ ID NO:4,
- (7) The DNA according to (5), which comprises a nucleotide sequence represented by SEQ ID NO:8,
- 15 (8) A recombinant vector comprising the DNA according to (5),
- (9) A transformant carrying the recombinant vector according to (8),
- (10) A process for producing a protein or a salt 20 thereof according to (1), which comprises culturing a transformant according to (9) under conditions suitable to express the protein,
- (11) A pharmaceutical composition which comprises the protein according to (1) or the partial peptide according to (4),
- 25 (12) The pharmaceutical composition according to (11) which is a therapeutic or prophylactic composition for diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk,
- (13) A pharmaceutical composition which comprises the DNA according to (5),
- (14) The pharmaceutical composition according to (13) 30 which is a therapeutic or prophylactic composition for diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis,
- 35

osteopetrosis or herniated disk,

(15) An antibody against the protein according to (1) or the partial peptide according to (4),

(16) A method for screening for a compound which activates or inhibits a proteolytic activity of the protein according to (1) or the partial peptide according to (4), which comprises measuring and comparing a proteolytic activity of the protein according to (1) or the partial peptide according to (4), in case of (i) a substrate is contacted with the protein according to (1) or the partial peptide according to (4) and (ii) a substrate and a test compound are contacted with the protein according to (1) or the partial peptide according to (4),

(17) A kit for screening for a compound which activates or inhibits a proteolytic activity of the protein according to (1) or the partial peptide according to (4), which comprises the protein according to (1) or the partial peptide according to (4),

(18) A compound which activates or inhibits a proteolytic activity of the protein according to (1) or the partial peptide according to (4), which is identified by the screening method according to (16) or the kit according to (17),

(19) A pharmaceutical composition which comprises the compound which inhibits a proteolytic activity of the protein according to (1) or the partial peptide according to (4), which is identified by the screening method according to (16) or the kit according to (17),

(20) A method for treating or preventing diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk in a mammal, which comprises administering an effective amount of the protein according to (1) or the partial peptide according to (4),

(21) Use of the protein according to (1) or the partial peptide according to (4) for production of a therapeutic or prophylactic composition for diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk,

5 (22) A method for treating or preventing diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk in a mammal, which comprises administering an effective amount of the DNA according to (5) to the mammal, and

10 (23) Use of the DNA according to (5) for production of a therapeutic or prophylactic composition for diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk.

Moreover, the present invention provides:

20 (24) The partial peptide according to (4), which comprises an amino acid sequence represented by any one of SEQ ID NO:3 to SEQ ID NO:6 or a substantial equivalent thereto,

25 (25) An isolated DNA which hybridizes under highstringent condition to a DNA comprising a nucleotide sequence represented by SEQ ID NO:7 or SEQ ID NO:8,

(26) A recombinant vector comprising the DNA according to (25),

30 (27) A transformant carrying the recombinant vector according to (26),

(28) A process for producing a protein which is encoded by the DNA according to (25) or a salt thereof comprising culturing a transformant according to (27) under conditions suitable to express the protein,

35 (29) A protein produced by the process according to (28),

(30) The pharmaceutical composition according to (19) which is a therapeutic or prophylactic composition for wound, rheumatoid arthritis, osteoarthritis, cancer (metasasis and invasion), periontitis, corneal ulcer, 5 gastric ulcer, myocardiopathy, aneurysm, otosclerosis, epidermolysis bullosa, premature delivery, atherosclerosis, septemia, multiple (disseminated) sclerosis, cachexia, hypercalcemia, leulemia, lymphoma, diabetes, systemic lupus erythematosus, asthma, 10 allergic rhinitis, atopic dermatitis, trauma, burn, acute pancreatitis, ischemia-reperfusion syndrome, myocardial infarction, congestive heart failure, organ transplantation or graft-vs.-host disease (GVHD),

(31) A pharmaceutical composition which comprises the 15 compound which activates a proteolytic activity of the protein according to (1) or the partial peptide according to (4), or a salt thereof, which is identified by the screening method according to (16) or the kit according to (17),

(32) The pharmaceutical composition according to (31) which is a therapeutic or prophylactic composition for diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk, 20

(33) A method of quantitative determination of the protein according to (1) or the partial peptide according to (4) in a test liquid sample, which comprises 25

(a) competitively reacting the test liquid sample and a labeled protein according to (1) or partial peptide according to (4) with the antibody according to (15), and

(b) measuring the ratio of the labeled protein according to (1) or partial peptide according to (4) 30

35 which binds to the antibody, and

(34) A method of quantitative determination of the

protein according to (1) or the partial peptide according to (4) in a test liquid sample, which comprises

5 (a) reacting the test liquid sample with the antibody according to (15) immobilized on an insoluble carrier and a labeled antibody according to (15) simultaneously or continuously, and

(b) measuring the activity of the labeling agent on the insoluble carrier.

10

The protein comprising the amino acid sequence represented by SEQ ID NO:1 (Fig. 1) or a substantial equivalent thereto of the present invention (hereinafter referred to as the protein of the present invention) may be (1) a protein derived from cells of human and other warm-blooded animals (e.g. guinea pig, rat, mouse, chicken, rabbit, swine, sheep, bovine, monkey, etc.) such as liver cell, splenocytes, nerve cell, glia cell, B cell, bone marrow cell, mesangial cell, Langerhans' cell, epidermic cell, epithelial cell, endothelial cell, fibroblast, fibrocyte, myocyte, fat cell, immune cell (e.g. macrophage, T cell, B cell, natural killer cell, mast cell, neutrophil, basophil, eosinophil, monocyte), megakaryocyte, synovial cell, chondrocyte, bone cell, osteoblast, osteoclast, mammary gland cell, hepatocyte, interstitial cell, etc., the corresponding precursor cells, stem cells, cancer cells, etc., or any tissues where such cells are present, such as brain or any of its regions (e.g. olfactory bulb, amygdaloid nucleus, basal ganglia, hippocampus, thalamus, hypothalamus, cerebral cortex, medulla oblongata, cerebellum, etc.), spinal cord, hypophysis, stomach, pancreas, kidney, liver, gonad, thyroid, gall-bladder, bone marrow, adrenal gland, 15 skin, muscle, lung, gastrointestinal tract (e.g. large intestine and small intestine), blood vessel, heart, 20 25 30 35

thymus, spleen, submandibular gland, peripheral blood, prostate, testis, ovary, placenta, uterus, bone, joint, skeletal muscle, etc., (2) a protein derived from cultured human cell lines (e.g. MEL, M1, CTLL-2, HT-2, 5 WEHI-3, HL-60, JOSK-1, K562, ML-1, MOLT-3, MOLT-4, MOLT-10, CCRF-CEM, TALL-1, Jurkat, CCRT-HSB-2, KE-37, SKW-3, HUT-78, HUT-102, H9, U937, THP-1, HEL, JK-1, CMK, KO-812, MEG-01, etc.), or (3) a synthetic protein.

Examples of the substantially equivalent amino acid sequence to the amino acid sequence represented by SEQ ID NO:1 are an amino acid sequence of not less than about 50%, preferably not less than about 60%, more preferably not less than about 70%, further more preferably not less than about 80%, for still better 10 result, not less than about 90%, best example, not less than about 95% identity to the amino acid sequence represented by SEQ ID NO:1 and so on. More preferable examples are an amino acid sequence of not less than about 50%, preferably not less than about 60%, more 15 preferably not less than about 70%, further more preferably not less than about 80%, for still better result, not less than about 90%, best example, not less than about 95% identity to the amino acid sequence represented by SEQ ID NO:1, which comprises at least an 20 amino acid sequence represented by SEQ ID NO:3 (Fig. 2) or SEQ ID NO:4 (Fig. 3), and so on.

Examples of the substantially equivalent amino acid sequence to the amino acid sequence represented by SEQ ID NO:1 are an amino acid sequence represented by 25 SEQ ID NO:2, and so on.

Examples of the protein comprising a substantially equivalent to the amino acid sequence represented by SEQ ID NO:1 are a protein which comprises a substantially equivalent amino acid sequence to the 30 amino acid sequence represented by SEQ ID NO:1 and has a substantially equivalent activity to the protein 35

comprising the amino acid sequence represented by SEQ ID NO:1, and so on.

More preferable examples of the protein are a protein comprising an amino acid sequence represented by SEQ ID NO:2, and so on.

Examples of the substantially equivalent activity are a proteolytic activity (e.g. activity of proteases such as proteinases, peptidases, etc.) and so on. The term "substantially equivalent" means that the nature of these activities are physiologically chemically or pharmacologically equivalent. Therefore, it is preferred that the strength of activities such as a proteolytic activity is equivalent (e.g. about 0.01 to 100 times, preferably about 0.5 to 20 times, more preferably about 0.5 to 2 times), and it is allowable that even differences among grades such as the strength of these activities and molecular weight of the protein are present.

Activities such as a proteolytic activity may be measured by per se known methods. For example, the proteolytic activity may be measured by the method for screening as mentioned below.

The proteins of the present invention include muteins such as proteins comprising (1) an amino acid sequence wherein 1 or more amino acid residues (for example 1 to 30, preferably 1 to 10, more preferably a few (1 to 5) amino acid residues) are deleted from the amino acid sequence represented by SEQ ID NO:1, (2) an amino acid sequence wherein 1 or more amino acid residues (for example 1 to 30, preferable 1 to 10, more preferable a few (1 to 5) amino acid residues) are added to the amino acid sequence represented by SEQ ID NO:1, (3) an amino acid sequence wherein 1 or more than acid residues (for example 1 to 30, preferably 1 to 10, more preferably a few (1 to 5) amino acid residues) in the amino acid sequence represented by SEQ ID NO:1 are

substituted with 1 or more amino acid residues (for example 1 to 30, preferably 1 to 10, more preferably a few (1 to 5) amino acid residues), or (4) combinations thereof.

5 When the amino acid sequence of the proteins are deleted or substituted as mentioned above, examples of the positions of deletion or substitution are, for example, (1) other than 98th to 508th amino acid sequence of the amino acid sequence represented by SEQ 10 ID NO:1 (other than an amino acid sequence represented by SEQ ID NO:3), more preferably, other than 93rd to 508th amino acid sequence of the amino acid sequence represented by SEQ ID NO:1 (an amino acid sequence represented by SEQ ID NO:4), or (2) other than 99th to 15 517th amino acid sequence of the amino acid sequence represented by SEQ ID NO:2 (other than an amino acid sequence represented by SEQ ID NO:5), more preferably, other than 94th to 517th amino acid sequence of the amino acid sequence represented by SEQ ID NO:2 (other 20 than an amino acid sequence represented by SEQ ID NO:6). Other preferable examples of the positions of deletion or substitution are other than a common sequence of the amino acid sequence represented by SEQ ID NO:1 and the amino acid sequence represented by SEQ 25 ID NO:2, and more preferable examples are, for example, other than 212nd to 225th amino acid sequence of the amino acid sequence represented by SEQ ID NO:1 (that is, other than 213rd to 226th amino acid sequence of the amino acid sequence represented by SEQ ID NO:2).

30 Throughout this specification, proteins are represented in accordance with the conventions for description of peptides, that is the N-terminus (amino terminus) at left and the C-terminus (carboxyl terminus) at right. The protein of the present 35 invention including the protein containing the amino acid sequence of SEQ ID NO:1 is usually in the carboxyl

(-COOH) or carboxylate (-COO⁻) form at the C-terminus but may be in the amide (-CONH₂) or ester (-COOR) form.

R in the ester residue includes a C₁₋₆ alkyl group (e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, etc., a C₃₋₈ cycloalkyl group (e.g. cyclopentyl, cyclohexyl, etc.), a C₆₋₁₂ aryl group (e.g. phenyl, α -naphthyl, etc.), a C₇₋₁₄ aralkyl group such as a phenyl-C₁₋₂ alkyl group (e.g. benzyl, phenethyl, etc.) and α -naphthyl-C₁₋₂ alkyl, (e.g. α -naphthylmethyl, etc.), as well as pivaloyloxymethyl which is universally used for the production of esters for oral administration.

When the protein of the present invention has a carboxyl (or carboxylate) function in any position other than the C-terminus, the corresponding carboxamide or ester form is also included in the scope of the invention. The ester mentioned just above may be any of the esters mentioned for the C-terminal carboxyl function.

Furthermore, the protein of the present invention includes (1) the protein in which the N-terminal Met has been protected with a protective group (e.g. C₁₋₆ acyl such as formyl or C₁₋₅ alkyl-carbonyl such as acetyl, etc.), (2) the protein in which the N-terminal side of Glu has been cleaved in vivo to form pyroglutamic acid, (3) the protein in which the side chain of any relevant constituent amino acid (e.g. OH, COOH, NH₂, SH) has been protected by any protective group (e.g. C₁₋₆ acyl group such as formyl or C₁₋₅ alkyl-carbonyl group such as acetyl, etc.), and (4) the complex protein such as glycoproteins available upon attachment of sugar chains.

Preferable Examples of the proteins of the present invention are human metalloproteases such as a human liver-derived metalloprotease comprising an amino acid sequence represented by SEQ ID NO:1 (Fig. 1), rat

metalloproteases such as a rat liver-derived metalloprotease comprising an amino acid sequence represented by SEQ ID NO:2 (Fig. 6).

Examples of the partial peptide of the present invention are any partial peptides of the protein of the present invention as mentioned above which have a proteolytic activity. For example, the partial peptides include peptides comprising at least not less than about 20, preferably not less than about 50, more preferably not less than about 70, for still better result, not less than about 100, best result, not less than 200 amino acid residues of the amino acid sequence of the proteins of the present invention.

Preferable examples of the partial peptide of the present invention are (1) a peptide which comprises an amino acid sequence represented by SEQ ID NO:3 or SEQ ID NO:4, or a substantially equivalent thereto and has a substantially equivalent activity to the protein comprising the amino acid sequence represented by SEQ ID NO:1, (2) a peptide which comprises an amino acid sequence represented by SEQ ID NO:5 or SEQ ID NO:6, or a substantially equivalent thereto and has a substantially equivalent activity to the protein comprising the amino acid sequence represented by SEQ ID NO:2.

Examples of the substantially equivalent amino acid sequence to the amino acid sequence represented by SEQ ID NO:3 or SEQ ID NO:4 are an amino acid sequence of not less than about 50%, preferably not less than about 60%, more preferably not less than about 70%, further more preferably not less than about 80%, for still better result, not less than about 90%, best example, not less than about 95% identity to the amino acid sequence represented by SEQ ID NO:3 or SEQ ID NO:4.

Examples of the substantially equivalent amino

acid sequence to the amino acid sequence represented by SEQ ID NO:5 or SEQ ID NO:6 are an amino acid sequence of not less than about 50%, preferably not less than about 60%, more preferably not less than about 70%,
5 further more preferably not less than about 80%, for still better result, not less than about 90%, best example, not less than about 95% identity to the amino acid sequence represented by SEQ ID NO:5 or SEQ ID NO:6.

10 The amino acid sequence represented by SEQ ID NO:3 shows an amino acid sequence from ⁹⁸Tyr to ⁵⁰⁸Tyr of the amino acid sequence represented by SEQ ID NO:1, and the amino acid sequence represented by SEQ ID NO:4 shows an amino acid sequence from ⁹³Gln to ⁵⁰⁸Tyr of the amino acid sequence represented by SEQ ID NO:1. The both amino acid sequences show amino acid sequences of catalytic domain of the protein of the present invention, and show amino acid sequence of the mature protein of the protein of the present invention comprising the amino acid sequence represented by SEQ ID NO:1.

15 The amino acid sequence represented by SEQ ID NO:5 shows an amino acid sequence from ⁹⁹Tyr to ⁵¹⁷Tyr of the amino acid sequence represented by SEQ ID NO:12, and the amino acid sequence represented by SEQ ID NO:6 shows an amino acid sequence from ⁹⁴Gln to ⁵¹⁷Tyr of the amino acid sequence represented by SEQ ID NO:12. The both amino acid sequences show amino acid sequences of catalytic domain of the protein of the present invention, and show amino acid sequence of the mature protein of the protein of the present invention comprising the amino acid sequence represented by SEQ ID NO:2.

30 The protein of the present invention is expressed as the protein comprising the amino acid sequence represented by SEQ ID NO:1, and is cleaved in vivo at
35

the position of 1st to 97th or 1st to 92nd of the amino acid sequence represented by SEQ ID NO:1. And the peptide comprising 98th to 508th amino acid sequence (SEQ ID NO:3, Fig. 2) or 93rd to 508th amino acid sequence (SEQ ID NO:4, Fig. 3) of the amino acid sequence represented by SEQ ID NO:1, etc. show a proteolytic activity, etc. as a mature protein or active protein.

10 The protein of the present invention is expressed as the protein comprising the amino acid sequence represented by SEQ ID NO:2, and is cleaved in vivo at the position of 1st to 98th or 1st to 93rd of the amino acid sequence represented by SEQ ID NO:2. And the peptide comprising 98th to 508th amino acid sequence (SEQ ID NO:5) or 94th to 517th amino acid sequence (SEQ ID NO:6) of the amino acid sequence represented by SEQ ID NO:2, etc. show a proteolytic activity, etc. as a mature protein or active protein.

20 The term "substantially equivalent activity" has the same meaning as defined above. The "substantially equivalent activity" can be measured by the same method as mentioned above.

25 The partial peptide of the present invention may include peptides such as peptide comprising (1) an amino acid sequence wherein 1 or more amino acid residues (for example 1 to 30, preferably 1 to 10, more preferably a few (1 to 5) amino acid residues) are deleted from the amino acid sequence represented by SEQ ID NO:3 or SEQ ID NO:4, (2) an amino acid sequence wherein 1 or more amino acid residues (for example 1 to 30, preferable 1 to 10, more preferable a few (1 to 5) amino acid residues) are added to the amino acid sequence represented by SEQ ID NO:3 or SEQ ID NO:4, (3) an amino acid sequence wherein 1 or more amino acid residues (for example 1 to 30, preferably 1 to 10, more preferably a few (1 to 5) amino acid residues) in the

amino acid sequence represented by SEQ ID NO:3 or SEQ ID NO:4 are substituted with 1 or more amino acid residues (for example 1 to 30, preferably 1 to 10, more preferably a few (1 to 5) amino acid residues), or (4) combinations thereof.

Other partial peptide of the present invention may include peptides such as peptide comprising (1) an amino acid sequence wherein 1 or more amino acid residues (for example 1 to 30, preferably 1 to 10, more preferably a few (1 to 5) amino acid residues) are deleted from the amino acid sequence represented by SEQ ID NO:5 or SEQ ID NO:6, (2) an amino acid sequence wherein 1 or more amino acid residues (for example 1 to 30, preferably 1 to 10, more preferably a few (1 to 5) amino acid residues) are added to the amino acid sequence represented by SEQ ID NO:5 or SEQ ID NO:6, (3) an amino acid sequence wherein 1 or more amino acid residues (for example 1 to 30, preferably 1 to 10, more preferably a few (1 to 5) amino acid residues) in the amino acid sequence represented by SEQ ID NO:5 or SEQ ID NO:6 are substituted with 1 or more amino acid residues (for example 1 to 30, preferably 1 to 10, more preferably a few (1 to 5) amino acid residues), or (4) combinations thereof.

The peptide of the present invention is usually in the carboxyl (-COOH) or carboxylate (-COO⁻) form at the C-terminus, but may instead be in the amide (-CONH₂) or ester (-COOR) form as same as the protein of the present invention as mentioned above.

Furthermore, the partial peptide of the present invention includes (1) the peptide in which the N-terminal Met has been protected with a protective group, (2) the peptide in which the N-terminal side of Glu has been cleaved in vivo to form pyroglutamic acid, (3) the peptide in which the side chain or any relevant constituent amino acid has been protected by any

protective group, and (4) the complex peptide such as glycoproteins available upon attachment of sugar chains as same as the protein of the present invention as mentioned above.

5 The specific examples of the partial peptide of the present invention are a peptide comprising an amino acid sequence represented by SEQ ID NO:3 (Fig. 2) or SEQ ID NO:4 (Fig. 3), a peptide comprising an amino acid sequence represented by SEQ ID NO:5 or SEQ ID 10 NO:6, and so on.

The salt of the protein or the partial peptide of the present invention includes salts with physiologically acceptable bases, e.g. alkali metals or acids such as organic or inorganic acids, and is 15 preferably a physiologically acceptable acid addition salt. Examples of such salts are salts thereof with inorganic acids (e.g. hydrochloric acid, phosphoric acid, hydrobromic acid or sulfuric acid, etc.) and salts thereof with organic acids (e.g. acetic acid, 20 formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid or benzenesulfonic acid, etc..)

25 The protein or a salt thereof of the present invention can be produced from the tissues or cells of human or other warm-blooded animals by per se known purification technology or, as described hereinafter, by culturing a transformant carrying a DNA encoding the protein. It can also be produced in accordance with 30 the procedures for peptide synthesis which are described hereinafter.

When the protein of the present invention is produced from the tissues or cells of human or other warm-blooded animals, the tissues or cells of human or 35 other warm-blood animals are homogenized and the protein of the present invention is extracted by an

acid, etc.. The protein can be purified and isolated from the extracted solution by a combination of chromatography such as reverse phase chromatography, ion exchange chromatography and so on.

5 For the synthesis of the protein of the present invention, a partial peptide thereof or their salts, or their amides form, any of commercial resins available for protein synthesis can be employed. Among such resins are chloromethyl resin, hydroxymethyl resin,
10 benzhydryl amino resin, aminomethyl resin, 4-benzyloxybenzyl alcohol resin, 4-methylbenzhydryl amino resin, PAM resin, 4-hydroxymethyl-methylphenylacetamidomethyl resin, polyacrylamide resin, 4-(2',4'-dimethoxyphenyl-hydroxymethyl)phenoxy resin, and 4-(2',4'-dimethoxyphenyl-Fmoc-
15 aminoethyl)phenoxy resin. Using such a resin, amino acids which may be beforehand protected at side-chain functional groups in a suitable manner can be serially condensed with the α -amino group in the order
20 corresponding to the amino acid sequence of the objective protein by various condensation techniques which are per se known. After completion of the final condensation reaction, the protein is separated from the resin and the protective groups are removed. Then,
25 in highly diluted solution, the intramolecular disulfide-forming reaction is carried out to provide the objective proteins or amides thereof.

Referring to the above condensation of protected amino acids, various activating agents known to be
30 useful for protein synthesis can be utilized, and carbodiimide reagents are especially preferred. The carbodiimide reagents include are DCC, N,N'-diisopropylcarbodiimide, and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide and so on. For activation by these reagents, the protected amino acid
35 and a racemization inhibitor (e.g. HOBt, HOOBt, etc.)

can be directly added to the resin, or the protected amino acid can be activated beforehand in the form of symmetric acid anhydride, HOBt ester or HOOBt ester and, then, added to the resin.

5 The solvent used for the above-mentioned activation of protected amino acids or the conjugation thereof to the resin can be optionally selected from among the solvents known to be useful for protein condensation reactions. Examples of the solvent are

10 acid amides (e.g. N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidone, etc.), halogenated hydrocarbons (e.g. methylene chloride, chloroform, etc.), alcohols (e.g. trifluoroethanol, sulfoxides (e.g. dimethyl sulfoxide, etc.), ethers

15 (e.g. pyridine, dioxane, tetrahydrofuran, etc.), nitriles (e.g. acetonitrile, propionitrile, etc.), esters (e.g. methyl acetate, ethyl acetate, etc.), and suitable mixtures of these solvents. The reaction temperature can be selected from the range known to be

20 useful for protein-forming reactions, usually the range of about -20°C to about 50°C. The activated amino acid derivative is generally used in a 1.5 to 4-fold excess. When the condensation is found insufficient by ninhydrin assay, the reaction can be repeated to make

25 the sufficient condensation thorough. When sufficient condensation can not be achieved by repeated reaction, an unreacted amino acid can be acetylated by using acetic anhydride or acetylimidazole so as not to effect a subsequent reaction.

30 The protective groups for protecting the amino group of the starting compound include Z, Boc, t-pentyloxycarbonyl, isobornyloxycarbonyl, 4-methoxybenzyloxycarbonyl, Cl-Z, Br-Z, adamantlyloxycarbonyl, trifluoroacetyl, phthaloyl, formyl, 2-

35 nitrophenylsulfenyl, diphenylphosphinothioly, Fmoc, and so on.

The carboxyl group can be protected in the form of, for example, an alkyl ester (e.g. straight-chain, branched, or cyclic alkyl esters such as methyl, ethyl, propyl, butyl, t-butyl, cyclopentyl, cyclohexyl, 5 cycloheptyl, cyclooctyl, 2-adamantyl, and so on), an aralkyl ester (e.g. benzyl, 4-nitrobenzyl, 4-methoxybenzyl, 4-chlorobenzyl, benzhydryl, and so on), phenacyl ester, benzyloxycarbonylhydrazide, t-butoxycarbonylhydrazide or tritylhydrazide.

10 The hydroxyl group of serine can be protected in the form of an ester or an ether. The group suitable for esterification includes carboxylic acid-derived acyl groups such as a lower(C₁₋₆) alkanoyl group (e.g. acetyl, etc.), an aroyl group (e.g. benzoyl, etc.), a 15 benzyloxycarbonyl, an ethoxycarbonyl group and so on. The group suitable for etherification includes a benzyl group, a tetrahydropyranyl group, a t-butyl group and so on.

20 The protective group used for protecting the phenolic hydroxyl group of tyrosine includes Bzl, Cl₂-Bzl, 2-nitrobenzyl, Br-Z, t-butyl and so on.

25 The protective group for the imidazole group of histidine includes Tos, 4-methoxy-2,3,6-trimethylbenzenesulfonyl, DNP, benzyloxymethyl, Bum, Boc, Trt, Fmoc and so on.

30 The starting compound with activated carboxyl groups includes the corresponding acid anhydride, azide, and active ester (e.g. esters with alcohols such as pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethyl alcohol, p-nitrophenol, HONB, N-hydroxysuccinimide, N-hydroxyphthalimide, HOBr, etc.). The starting compound with activated amino groups includes the corresponding phosphoryl amide.

35 The method for removal of such protective groups includes catalytic reduction in a hydrogen stream in the presence of a catalyst (e.g. Pd black or Pd-on-

carbon), acid treatment with anhydrous hydrogen fluoride, methanesulfonic acid, trifluoromethanesulfonic acid, trifluoroacetic acid or a mixture thereof, treatment with a base such as diisopropylethylamine, triethylamine, piperidine, piperazine or the like, and reduction with sodium metal in liquid ammonia. The above deprotection by treatment with acid is generally conducted at a temperature of about -20°C to 40°C. This acid treatment can be carried out advantageously in the presence of a cation acceptor such as anisole, phenol, thioanisole, m-cresol, p-cresol, dimethyl sulfide, 1,4-butanedithiol, 1,2-ethanedithiol, or the like. The 2,4-dinitrophenyl group used for protecting the imidazole group of histidine can be removed by treatment with thiophenol, and the formyl group used for protecting the indole group of tryptophan can be removed not only by said acid treatment in the presence of 1,2-ethanedithiol, 1,4-butanedithiol or the like as described hereinbefore, but also by alkali treatment with diluted sodium hydroxide solution, diluted liquid ammonia, or the like.

The method for protecting any functional group that should not take part in the contemplated reaction, the protective group to be used for such protection, the method for eliminating the protective group, and the method for activating the functional group to be involved in the contemplated reaction can all be optionally selected from among the known methods and groups.

An alternative method for providing the protein in amide form typically comprises protecting the α -carboxyl group of the C-terminal amino acid in the form of an amide, extending the peptide (protein) chain to a desired length towards the N-terminus, deprotecting the N-terminal α -amino acid of the resulting peptide chain

selectively to provide an N-terminal-deprotected fragment, preparing a peptide (protein) fragment with its C-terminal carboxyl group selectively deprotected, and condensing the two fragments in a solvent such as 5 the mixed solvent as mentioned above. The condensation reaction can be carried out in the same manner as described hereinbefore. After purification of the protected protein thus obtained by condensation, all the protective groups are eliminated by the procedures 10 described hereinbefore to provide the contemplated protein in crude form. This crude protein is purified by suitable known purification techniques and lyophilized to provide the desired protein amide.

15 A method for providing the protein in an ester form comprises condensing the α -carboxyl group of the C-terminal amino acid with a suitable alcohol to prepare the corresponding ester and subjecting this ester to the same procedure as described for 20 purification of the protein amide to provide the objective protein ester.

25 The partial peptide of the protein of the present invention or a salt thereof can be produced by *per se* known procedures for peptide synthesis or by cleaving the protein with a suitable peptidase. The process for peptide synthesis may be a solid-phase synthesis and/or a liquid-phase synthesis. Namely, the objective peptide can be produced by condensing a partial peptide 30 or amino acid capable of constituting the protein with the residual part thereof and, when the product has a protective group, the protective group is removed whereupon a desire peptide can be manufactured. The known technology for condensation and deprotection includes the procedures described in the following literature (1)-(5).

35 (1) M. Bodanszky and M. A. Ondetti, *Peptide Synthesis*, Interscience Publishers, New York, 1966

(2) Schroeder and Luebke, *The Peptide*, Academic Press, New York, 1965

(3) Nobuo Izumiya et al., *Fundamentals and Experiments in Peptide Synthesis*, Maruzen, 1975

5 (4) Haruaki Yajima and Shumpei Sakakibara, *Biochemical Experiment Series 1, Protein Chemistry IV*, 205, 1977

(5) Haruaki Yajima (ed.), *Development of Drugs-Continued, 14, Peptide Synthesis*, Hirokawa Shoten

10 After the reaction, the partial peptide of the present invention can be purified and isolated by a combination of conventional purification techniques such as solvent extraction, distillation, column chromatography, liquid chromatography, and recrystallization. When the partial peptide isolated
15 as above is in a free form, it can be converted to a suitable salt by known methods or method analogous thereto. On the other hand, when it is isolated as a salt, it can be converted to a free form or to any other salt thereof by known methods or method analogous
20 thereto.

25 The DNA coding for the protein of the present invention may be any DNA comprising a nucleotide sequence encoding the protein of the present invention as mentioned above. It may also be any one of genomic DNA, genomic DNA library, cDNA derived from the tissues or cells as mentioned above, cDNA library derived from the tissues or cells as mentioned above, and synthetic DNA.

30 The vector for constructing a library may include bacteriophage, plasmid, cosmid, and phagemid. Furthermore, using a totalRNA fraction or an mRNA fraction prepared from the tissues or cells, a direct amplification can be carried out by Reverse Transcriptase Polymerase Chain (hereinafter, referred
35 to as RT-PCR method) technique.

Examples of DNA coding for the protein of the

present invention are (1) a DNA comprising a nucleotide sequence represented by SEQ ID NO:7, or a DNA which comprises a nucleotide sequence hybridizing to the nucleotide sequence represented by SEQ ID NO:7 under a highstringent condition and codes for a protein having a substantially equivalent activity to the protein comprising the amino acid sequence represented by ID No:1, (2) a DNA comprising a nucleotide sequence represented by SEQ ID NO:8, or a DNA which comprises a nucleotide sequence hybridizing to the nucleotide sequence represented by SEQ ID NO:8 under a highstringent condition and codes for a protein having a substantially equivalent activity to the protein comprising the amino acid sequence represented by ID No:2, and so on.

Examples of the nucleotide sequence hybridizing to the nucleotide sequence represented by SEQ ID NO:7 are a nucleotide sequence of not less than about 70%, preferably not less than about 80%, more preferably not less than about 90%, for still better result, not less than about 95% identity to the nucleotide sequence represented by SEQ ID NO:7.

Examples of the nucleotide sequence hybridizing to the nucleotide sequence represented by SEQ ID NO:8 are a nucleotide sequence of not less than about 70%, preferably not less than about 80%, more preferably not less than about 90%, for still better result, not less than about 95% identity to the nucleotide sequence represented by SEQ ID NO:8.

The hybridization can be carried out by per se known methods such as the method described in Molecular Cloning, 2nd (J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989) and so on. When a commercially available library is used, the hybridization can be carried out in accordance with the instructions given in the accompanying manual, and particularly, be

carried out under a highstringent condition.

Under the highstringent condition, Na^+ concentration is at about 19 to 40mM, preferably about 19 to 20 mM and a temperature is at about 50 to 70°C, 5 preferably about 60 to 65°C. Particularly, the condition at about 19 mM of Na^+ and about 65°C is preferred.

Preferable examples of the DNA coding for the protein comprising an amino acid sequence represented 10 by SEQ ID NO:1 are a DNA comprising the nucleotide sequence represented by SEQ ID NO:7 (95th to 1618th nucleotide sequence of the nucleotide sequence of Fig. 4), and preferable example of the DNA coding for the protein comprising an amino acid sequence represented 15 by SEQ ID NO:2 are a DNA comprising the nucleotide sequence represented by SEQ ID NO:8 (90th to 1640th nucleotide sequence of the nucleotide sequence of Fig. 6).

The DNA coding for the partial peptide of the 20 present invention may be any DNA comprising a nucleotide sequence encoding the partial peptide of the present invention as mentioned above. It may also be any one of genomic DNA, genomic DNA library, cDNA derived from the tissues or cells as mentioned above, 25 cDNA library derived from the tissues or cells as mentioned above, and synthetic DNA.

Examples of DNA coding for the partial peptide of the present invention are (1) a DNA comprising a 30 partial nucleotide sequence of DNA which comprises a nucleotide sequence represented by SEQ ID NO:7, or a DNA comprising a partial nucleotide sequence of DNA which comprises a nucleotide sequence hybridizing under a highstringent condition to the nucleotide sequence represented by SEQ ID NO:7 and codes for a protein 35 having a substantially equivalent activity to the protein comprising the amino acid sequence represented

by SEQ ID NO:1, (2) a DNA comprising a partial nucleotide sequence of DNA which comprises a nucleotide sequence represented by SEQ ID NO:8, or a DNA comprising a partial nucleotide sequence of DNA which 5 comprises a nucleotide sequence hybridizing under a highstringent condition to the nucleotide sequence represented by SEQ ID NO:8 and codes for a protein having a substantially equivalent activity to the protein comprising the amino acid sequence represented 10 by SEQ ID NO:2, and so on.

Preferable examples of DNA coding for the partial peptide of the present invention are (1) a DNA comprising a nucleotide sequence represented by SEQ ID NO:9, or a DNA which comprises a nucleotide sequence hybridizing under a highstringent condition to the nucleotide sequence represented by SEQ ID NO:9 and codes for a partial peptide having a substantially equivalent activity to the protein of the present 15 invention, (2) a DNA comprising a nucleotide sequence represented by SEQ ID NO:10, or a DNA which comprises a nucleotide sequence hybridizing under a highstringent condition to the nucleotide sequence represented by SEQ ID NO:10 and codes for a partial peptide having a substantially equivalent activity to the protein of the present 20 invention, (3) a DNA comprising a nucleotide sequence represented by SEQ ID NO:11, or a DNA which comprises a nucleotide sequence hybridizing under a highstringent condition to the nucleotide sequence represented by SEQ ID NO:11 and codes for a partial peptide having a substantially equivalent activity to the protein of the present 25 invention, (4) a DNA comprising a nucleotide sequence represented by SEQ ID NO:12, or a DNA which comprises a nucleotide sequence hybridizing under a highstringent condition to the nucleotide sequence represented by SEQ ID NO:12 and codes for a partial peptide having a substantially 30 equivalent activity to the protein of the present invention, (5) a DNA comprising a nucleotide sequence represented by SEQ ID NO:13, or a DNA which comprises a nucleotide sequence hybridizing under a highstringent condition to the nucleotide sequence represented by SEQ ID NO:13 and codes for a partial peptide having a substantially 35 equivalent activity to the protein of the present invention.

equivalent activity to the protein of the present invention, and so on.

Examples of the nucleotide sequence hybridizing to the nucleotide sequence represented by SEQ ID NO:9 or 5 SEQ ID NO:10 are a nucleotide sequence of not less than about 70%, preferably not less than about 80%, more preferably not less than about 90%, for still better result, not less than about 95% identity to the nucleotide sequence represented by SEQ ID NO:9 or SEQ 10 ID NO:10, and so on.

Examples of the nucleotide sequence hybridizing to the nucleotide sequence represented by SEQ ID NO:11 or SEQ ID NO:12 are a nucleotide sequence of not less than about 70%, preferably not less than about 80%, more 15 preferably not less than about 90%, for still better result, not less than about 95% identity to the nucleotide sequence represented by SEQ ID NO:11 or SEQ ID NO:12, and so on.

The method for hybridization and the highstringent 20 condition have same meanings as mentioned above.

Preferable examples of the DNA coding for the partial peptide represented by SEQ ID NO:3 are a DNA comprising the nucleotide sequence represented by SEQ 25 ID NO:9 (386th to 1618th nucleotide sequence of the nucleotide sequence of Fig. 4) and so on. Preferable examples of the DNA coding for the protein represented by SEQ ID NO:4 are a DNA comprising the nucleotide sequence represented by SEQ ID NO:10 (371th to 1618th nucleotide sequence of the nucleotide sequence of Fig. 30 4) and so on.

Preferable examples of the DNA coding for the partial peptide comprising an amino acid sequence represented by SEQ ID NO:5 are a DNA comprising the nucleotide sequence represented by SEQ ID NO:11 (295th 35 to 1640th nucleotide sequence of the nucleotide sequence of Fig. 6) and so on. Preferable examples of

the DNA coding for the protein comprising an amino acid sequence represented by SEQ ID NO:6 are a DNA comprising the nucleotide sequence represented by SEQ ID NO:12 (280th to 1640th nucleotide sequence of the 5 nucleotide sequence of Fig. 6) and so on.

The DNA encoding the entire protein or the partial peptide of the present invention can be cloned either by PCR amplification using synthetic DNA primers having a partial nucleotide sequence of the DNA coding for the 10 protein or by hybridization using the DNA inserted in a suitable vector and labeled DNA fragment or synthetic DNA coding for a part or full region of the protein or the partial peptide of the present invention. The hybridization can be carried out by the method 15 described in Molecular Cloning, 2nd (J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989). When a commercially available DNA library is used, the instructions given in the accompanying manual can be followed.

20 The substitution of the nucleotide sequence of the DNA can be carried out by the per se known method such as Gapped duplex method, Kunkel method and so on by using the known kits such as MutanTM-G (Takara corporation), MutanTM-K (Takara corporation) and so on.

25 The cloned DNA coding for the protein of the present invention can be used directly or after digestion with a restriction enzyme or after addition of a linker depending on purposes. This DNA may have 30 ATG as the translation initiation codon at the 5' end and TAA, TGA, or TAG as the termination codon at the 3' end. The translation initiation and termination codons can be added by means of suitable DNA adapters.

An expression vector for the protein of the 35 present invention can be produced by, for example, (a) cutting out an objective DNA fragment from the DNA for

the protein of the present invention and (b) ligating the objective DNA fragment with the downstream side of a promoter in a suitable expression vector.

The vector may include plasmids derived from 5 Escherichia coli, e.g., pBR322, pBR325, pUC12, pUC13, etc.; plasmids derived from Bacillus subtilis, e.g., pUB110, pTP5, pC194, etc.; plasmids derived from yeasts e.g., pSH19, pSH15, etc.; bacteriophages such as λ - 10 phage; animal virus such as retrovirus, vaccinia virus, etc.; insect virus; and other vectors such as pAl-11, pXT1, pRc/CMV, pRc/RSV, pcDNA1/Neo and so on.

According to the present invention, any promoter can be used as long as it is appropriate for the host cell which is used for expressing a gene. When the 15 host is an animal cell, the promoter include SR α promoter, SV40 promoter, LTR promoter, CMV (cytomegalovirus) promoter, HSV-TK promoter, etc., and CMV promoter and SR α promoter are preferably used. When the host for the transformation is 20 Escherichia coli, the promoter are preferably trp promoter, lac promoter, recA promoter, λ P_L promoter, lpp promoter, T7 promoter, etc.. When the host for the transformation is Bacillus, the promoter are preferably SPO1 promoter, SPO2 promoter, penP promoter, etc.. When the host is a 25 yeast, the promoter are preferably PHO5 promoter, PGK promoter, GAP promoter, ADH promoter, AOX1 promoter, etc.. When the host is an insect cell, the promoter include polyhedrin promoter, P10 promoter, etc..

The expression vectors may, if necessary, further 30 comprise enhancers, splicing signals, polyadenylation signals, selective markers, SV40 duplicate origin (hereinafter referred to as SV40 ori). Examples of selective markers are dihydrofolic acid reductase (hereinafter referred to as dhfr gene (methotrexate 35 (MTX) resistant)), neomycin-resistant gene (hereinafter referred to as Neo, G418 resistant) and so on.

Particularly, when the dhfr gene is used as a selective marker against gene-deficient chinese hamster cell lines, cells transfected by the objective gene can be selected in a thymidine-free medium.

5 Furthermore, an appropriate signal sequence for a host can be added to the N-terminal side of the protein. When the host is Escherichia coli, the utilizable signal sequences may include PhoA signal sequence, OmpA signal sequence, etc.. When the host is 10 Bacillus, they may include α -amylase signal sequence, subtilisin signal sequence, etc.. When the host is a yeast, they may include M α signal sequence, SUC2 signal sequence, etc.. When the host is an animal cell, they may include insulin signal sequence, α 15 -interferon signal sequence, antibody molecule signal sequence, etc..

A transformant or transfectant is produced by using the vector thus constructed, which carries the DNA coding for the protein of the present invention.

20 The host may be, for example, Escherichia species, Bacillus species, yeast cells, insect cells, insects, animal cells, etc..

25 Examples of Escherichia species include Escherichia coli K12-DH1 (Proceedings of the National Academy of Sciences of the United State of America, Vol. 60, 160 (1968)), JM103 (Nucleic Acids Research, Vol. 9, 309 (1981)), JA221 (Journal of Molecular Biology, Vol. 120, 517 (1978)), HB101 (Journal of molecular Biology, Vol. 41, 459 (1969)), C600 30 (Genetics, Vol. 39, 440 (1954)), etc..

Examples of Bacillus species are, for example, Bacillus subtilis M1114 (Gene, Vol. 24, 255 (1983)), 207-21 (Journal of Biochemistry, Vol. 95, 87 (1984)), etc..

35 Examples of yeast cells are, for example, Saccharomyces cerevisiae AH22, AH22R, NA87-11A, DKD-5D

or 20B-12, Schizosachcaromyces pombe NCYC1913 or Pichia pastoris, etc..

Examples of insect cells are, for example, Spodoptera frugiperda cell (Sf cell), MG1 cell derived 5 from center intestine of Trichoplusia ni, High FiveTM cell derived from eggs of Trichoplusia ni, Mamestra brassicae-derived cell, Estigmene acrea-derived cell and so on when virus is AcNPV; and Bombyx mori N cell (BmN cell) and so on when virus is BmNPV. Examples of 10 the Sf cell are, for example, Sf9 cell (ATCC CRL 1711), Sf21 cell [both, Vaughn J.L. et al., In Vivo, 13, 213-217(1977)] and so on.

Examples of insects include a larva of silkworm (Bombyx mori larva) (Maeda et al., Nature, 315, 15 592(1985)).

Examples of animal cells are, for example, monkey-derived COS-7 cell line, Vero cell line, Chinese hamster ovary cell line (hereinafter referred to as CHO cell), dhfr gene-deficient Chinese hamster cell line 20 (hereinafter referred to as CHO(dhfr⁻) cell), mouse L cell, mouse AtT-20, mouse myeloma cell, rat GH3, human FL, 293 cell, C127 cell, BALB3T3 cell, Sp-2/O cell, etc.. Among them, CHO cell, CHO(dhfr⁻) cell, 293 cell, etc.. are preferred.

Depending on host cells used, transformation is 25 done using standard techniques appropriate to such cells.

Transformation of Escherichia species can be 30 carried out in accordance with methods as disclosed in, for example, Proceedings of the National Academy of Sciences of the United State of America, Vol. 69, 2110 (1972), and Gene, Vol. 17, 107 (1982), etc..

Transformation of Bacillus species can be carried out 35 in accordance with methods as disclosed in, for example, Molecular & General Genetics, Vol. 168, 111 (1979), etc..

Transformation of yeast cells can be carried out in accordance with methods as disclosed in, for example, *Methods in Enzymology*, 194, 182-187(1991), etc.. Transformation of insect cells or insects can be 5 carried out in accordance with methods as disclosed in, for example, *Bio/Technology*, 6, 47-55, (1988).

Transformation of animal cells can be carried out by methods as disclosed in, for example, *Cell Engineering*, separate vol. 8, *New Cell Engineering* 10 *Experiment Protocol*, 263-267(1995) (Shujun Company), *Virology*, Vol. 52, 456 (1973), etc..

In introducing the expression vector into cells, known methods such as a calcium phosphate method (Graham, F. L. and van der Eb, A. J.: *Virology*, 52, 15 456-467(1973)), an electroporation (Neumann, E. et al., *EMBO Journal*, 1,841-845(1982)), etc. may be used.

The transformants or transfectants wherein the expression vector carrying the DNA coding for the protein can be obtained according to the aforementioned 20 techniques.

Examples of methods for expressing the protein of the present invention stably using animal cells are a method for selecting the cells wherein the above-mentioned expression vector is incorporated in the 25 chromosome by means of clone selection. Briefly, the transformant is first selected using the above-mentioned selective marker as an index for selection. Then the animal cell produced as such using the selective marker is repeatedly subjected to a clone 30 selection to give an animal cell strain which stably exhibits a high ability of expressing the protein of the present invention. When a dhfr gene is used as a selective marker, the resisting cells are selected by a culture with a sequential increase in the MTX 35 concentration to amplify the DNA coding for the protein of the present invention with dhfr gene in the cells

whereby an animal cell strain exhibiting far higher expression can be obtained.

5 The protein of the present invention or a salt thereof can be also manufactured by culturing the transformant under a condition where the DNA coding for the protein of the present invention can be expressed to express and accumulate the protein of the present invention.

10 Culture of the transformants (transfектанты) of Escherichia or Bacillus species can be carried out preferably in a liquid culture medium. The culture medium may contain carbon sources, nitrogen sources, minerals, etc. which are necessary for growing the transformants. The carbon sources may include glucose, 15 dextrin, soluble starch, sucrose, etc.. The nitrogen sources may include organic or inorganic substances such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extracts, bean-cakes, potato extracts, etc.. Examples of the minerals may include 20 calcium chloride, sodium dihydrogen phosphate, magnesium chloride, etc.. It is further allowable to add yeast extracts, vitamins, growth-promoting factors, etc.. It is suitable that the pH of culture medium is at about 5 to 8.

25 The culture medium for Escherichia species is, for example, preferably M9 medium which contains glucose and casamino acid (Miller, Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor Laboratory, New York, (1972)). If necessary, drugs such as 3 β -indolyl acrylic acid can be added to the medium to improve efficiency of the promoter. In the case of Escherichia organisms as a host, the culture is carried out usually at about 15 to 43°C for about 3 to 24 hours. As required, aeration and stirring may be 30 applied. In the case of Bacillus organisms as a host, the culture is carried out usually at about 30 to 40°C 35

for about 6 to 24 hours. As required, aeration and stirring may also be applied.

In the case of yeast transformants, the culture medium used may include, for example, Burkholder 5 minimum medium (Bostian, K.L. et al., Proceedings of the National Academy of Sciences of the United State of America, Vol. 77, 4505 (1980)), SD medium containing 0.5% casamino acid (Bitter, G.A. et al., Proceedings of the National Academy of Sciences of the United State of 10 America, Vol. 81, 5330 (1984)), etc.. It is preferable that the pH of the culture medium is adjusted to be from about 5 to 8. The culture is carried out usually at about 20 to 35°C for about 24 to 72 hours. As required, aeration and stirring may be applied.

15 In the case of the transformants (or transfectants) of insect cells or insects, the culture medium used may include the Grace's insect medium supplemented with additives such as inactivated 10% bovine serum (Grace, T.C.C., Nature, 195, 788 (1962)). 20 It is preferable that the pH of the culture medium is adjusted to be about 6.2 to 6.4. The culture is usually carried out at about 27°C for about 3 to 5 days. As desired, aeration and stirring may be applied.

25 In the case of the transformants (or transfectants) of animal cells, the culture medium used may include MEM medium (Science, Vol. 122, 501 (1952)), DMEM medium (Virology, Vol. 8, 396 (1959)), RPMI 1640 medium (Journal of the American Medical Association, 30 Vol. 199, 519 (1967)), 199 medium (Proceedings of the Society of the Biological Medicine, Vol. 73, 1 (1950)), etc. which are containing, for example, about 5 to 20% of fetal calf serum. It is preferable that the pH is from about 6 to 8. The culture is usually carried out 35 at about 30 to 40°C for about 15 to 72 hours. As required, medium exchange, aeration and stirring may be

applied. Especially when CHO (dhfr⁻) cells and dhfr selective marker gene are used, it is preferred to use a DMEM medium containing a dialyzed fetal bovine serum which rarely contains thymidine.

5 Separation and purification of the protein from the above-mentioned cultures can be carried out according to methods described herein below.

10 To extract the protein from the cultured microorganisms, insects or cells, the microorganisms or cells are collected by known methods after the culture, suspended in a suitable buffer solution, disrupted by ultrasonic waves, lysozyme and/or freezing and thawing, etc. and, then, a crude protein extract is obtained by centrifugation or filtration. Other conventional 15 extraction or isolation methods can be applied. The buffer solution may contain a protein-denaturing agent such as urea or guanidine hydrochloride or a surfactant such as Triton X-100TM.

20 In the case where proteins are secreted into culture media, supernatants are separated from the microorganisms or cells after culture and collected by known methods. The culture supernatant containing the protein can be purified by suitable combinations of known methods for separation, isolation and 25 purification. The known methods of separation, isolation and purification may include methods which utilizes solubility, such as salting out or sedimentation with solvents, methods which utilizes chiefly a difference in the molecular size or weight, 30 such as dialysis, ultrafiltration, gel filtration and SDS-polyacrylamide gel electrophoresis, methods utilizing a difference in the electric charge, such as ion-exchange chromatography, methods utilizing specific affinity such as affinity chromatography, methods 35 utilizing a difference in the hydrophobic property, such as reversed-phase high-performance liquid

chromatography, and methods utilizing a difference in the isoelectric point such as isoelectric electrophoresis, etc..

5 In cases where the protein thus obtained is in a free form, the free-form protein can be converted to a salt thereof by known methods or method analogous thereto. In case, where the protein thus obtained is in a salt form vice versa, the protein salt can be converted to a free form or to any other salt thereof by known methods or method analogous thereto.

10 The protein produced by the transformant can be arbitrarily modified or a polypeptide can be partly removed therefrom, by a suitable protein-modifying enzyme before or after the purification. The protein-modifying enzyme may include trypsin, chymotrypsin, 15 arginyl endopeptidase, protein kinase, glycosidase, etc.. The activity of the protein of the present invention thus obtained can be measured by binding assay with a labeled ligand or by enzyme immunoassays 20 (enzyme linked immunoassays) using specific antibodies.

25 The antibodies against the protein of the present invention, its partial peptide or a salt of either of them are any antibodies such as polyclonal antibodies and monoclonal antibodies which can recognize the protein of the present invention, its partial peptide or a salt of either of them.

30 The antibodies against the protein of the present invention, its partial peptide or a salt of either of them (hereinafter referred to as the protein of the present invention) may be manufactured by methods per se known to those of skill in the art or methods similar thereto, using the protein of the present invention as antigen. For example, polyclonal 35 antibodies can be manufactured by the method as given below.

Preparation of Monoclonal Antibody:

(a) Preparation of Monoclonal Antibody-Producing Cells

The protein of the present invention is administered to warm-blooded animals either solely or 5 together with carriers or diluents to the site favorable for antibody production. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvants or incomplete Freund's adjuvants may be administered. The 10 administration is usually carried out once every 2 to 6 weeks and 2 to 10 times in total. Examples of the applicable warm-blooded animals are monkeys, rabbits, dogs, guinea pigs, mice, rats, sheep, goats and chickens. The use of mice and rats is preferred.

15 In establishing cells which produce monoclonal antibodies, an animal with the detectable antibody titer is selected from animals (e.g. mice) immunized with antigens, then spleen or lymph node is collected after 2 to 5 days from the final immunization and 20 antibody-producing cells contained therein are fused with myeloma cells derived from homogeneous or heterogeneous animals to obtain monoclonal antibody-producing hybridomas. Measurement of the antibody titer in antisera may, for example, be carried out by 25 reacting a labeled protein, which will be mentioned later, with the antiserum followed by measuring the binding activity of the labeling agent with the antibody. The cell fusion may be carried out, for example, by a method of Kohler and Milstein (Nature, 30 256, 495, 1975). Examples of the fusion accelerator are polyethylene glycol (PEG), Sendai virus, etc. and the use of PEG is preferred.

35 Examples of the myeloma cells are those derived from warm-blooded animals such as NS-1, P3U1, SP2/0, AP-1, etc. and the use of P3U1 is preferred. The preferred fusion ratio of the numbers of antibody-

producing cells used (spleen cells) to the numbers of myeloma cells is within a range of about 1:1 to 20:1. When PEG (preferably, PEG 1000 to PEG 6000) is added in a concentration of about 10 to 80% followed by 5 incubating at 20 to 40°C, preferably, at 30 to 37°C, for 1 to 10 minutes, an efficient cell fusion can be carried out.

Various methods may be applied for screening a hybridoma which produces a monoclonal antibody. For 10 example, a supernatant of hybridoma culture is added to a solid phase (e.g. microplate) to which the protein antigen is adsorbed either directly or with a carrier, then anti-immunoglobulin antibody (anti-mouse 15 immunoglobulin antibody is used when the cells used for the cell fusion are those of mouse) which is labeled with a radioactive substance, an enzyme or the like, or protein A is added thereto and then monoclonal 20 antibodies bound on the solid phase are detected; or a supernatant of the hybridoma culture is added to the solid phase to which anti-immunoglobulin or protein A is adsorbed, then the protein labeled with a radioactive substance or an enzyme is added and 25 monoclonal antibodies bound with the solid phase is detected.

Selection and cloning of the monoclonal antibody-producing hybridoma may be carried out by methods per se known to those of skill in the art or methods similar thereto. Usually, it is carried out in a 30 medium for animal cells, containing HAT (hypoxanthine, aminopterin and thymidine). With respect to a medium for the selection, for the cloning and for the growth, any medium may be used so far as hybridoma is able to grow therein. Examples of the medium are an RPMI 1640 medium (Dainippon Pharmaceutical Co., Ltd., Japan) 35 containing 1 to 20% (preferably 10 to 20%) of fetal calf serum (FCS), GIT medium (Wako Pure Chemical,

Japan) containing 1 to 20% of fetal calf serum and a suitable serum-free medium for hybridoma (SFM-101; Nissui Seiyaku, Japan). The culture temperature is usually 20 to 40°C and, preferably, about 37°C. The 5 culture period is usually from five days to three weeks and, preferably, one to two weeks. The culture is usually carried out in 5% carbon dioxide gas. The antibody titer of the supernatant of the hybridoma culture may be measured by the same manner as in the 10 above-mentioned measurement of the antibody titer in the antiserum.

(b) Purification of the Monoclonal Antibody

The separation and purification of the monoclonal antibody may be carried out by methods for 15 separating/purifying immunoglobulin such as salting-out, precipitation with alcohol, isoelectric precipitation, electrophoresis, adsorption/deadsorption using ion exchangers such as DEAE, ultracentrifugation, gel filtration, specific purifying methods in which 20 only an antibody is collected by treatment with an active adsorbent such as an antigen-binding solid phase, protein A or protein G and the bond is dissociated whereupon the antibody is obtained.

Preparation of a polyclonal antibody:

25 The polyclonal antibody of the present invention can be produced by per se known methods or methods analogous thereto. The method comprises preparing an immunogen (antigen protein) per se or a conjugate of an immunogen with a carrier protein, immunizing a warm-blooded animal in the same manner as described for the 30 production of the monoclonal antibody, harvesting a fraction containing the antibody against the protein of the present invention from the immunized animal, and purifying the harvested antibody.

35 Referring to the immunogen-carrier protein conjugate for use in the immunization of a warm-blooded

animal, the kind of carrier protein and the ratio of the carrier and hapten are not particularly restricted only if the production of the antibody against the hapten conjugated with the particular carrier protein and used for immunization proceeds efficiently. Thus, for example, bovine serum albumin, bovine thyroglobulin, hemocyanine, or the like is coupled in the weight ratio of about 0.1 to 20, preferably about 1 to about 5, to unity of the hapten.

A variety of condensing agents can be used for this coupling between the hapten and the carrier. Thus, for example, a glutaraldehyde, carbodiimide, maleimide, or a thiol or dithiopyridyl group-containing active ester reagent can be employed.

The condensation reaction product is administered to a warm-blooded animal at a site favorable for antibody production, either as it is alone or together with a carrier or diluent. Enhancing antibody production, complete Freund's adjuvant or incomplete Freund's adjuvant may be administered. Administration is carried out generally once in about 2 to 6 weeks for a total of about 3 to 10 times.

The polyclonal antibody can be harvested from the blood, ascites fluid, or other body fluid, preferably from the blood, of the host warm-blooded animal.

The polyclonal antibody titer in the antiserum can be determined in the same manner as the determination of monoclonal antibody described hereinbefore. The separation and purification of the polyclonal antibody can be carried out by the same method as that described for the separation and purification of monoclonal antibody.

The antisense DNA having a nucleotide sequence complementary or substantially complementary to the DNA coding for the protein or the partial peptide of the

present invention (hereinafter referred to as the DNA of the present invention) can be any antisense DNA having a nucleotide sequence complementary or substantially complementary to that of the DNA of the 5 present invention and capable of suppressing expression of the DNA.

The nucleotide sequence substantially complementary to the DNA of the present invention may, for example, be a nucleotide sequence having an 10 identity of not less than about 70%, preferably not less than about 80%, more preferably not less than about 90%, and for still better results, not less than about 95% to the total nucleotide sequence or partial nucleotide sequence of the nucleotide sequence 15 complementary to that of the DNA of the present invention. Particularly preferred is an antisense DNA having an identity of not less than about 70%, preferably not less than about 80%, and more preferably not less than about 90%, and for still better results, not less than about 95% to the nucleotide sequence of the domain, of the complete nucleotide sequence complementary to that 20 of the DNA of the invention, which encodes the N-terminal region of the protein of the present invention (e.g. the nucleotide sequence of the domain around the initiation codon). The antisense DNA can be 25 synthesized using a known DNA synthesis hardware.

The protein of the present invention is a 30 metalloprotease (preferably human-liver metalloprotease) such that the molecular weight of its proteinaceous domain is about 2 to 7×10^4 Da, preferably about 2 to 6×10^4 Da, and the molecular weight of its proteolytic domain is about 2 to 5×10^4 Da, the activity of which is elevated in, for example, 35 ovulation, development and differentiation, osteogenesis, atretic uterus, and vascularization.

Moreover, its activity is increased in, for example, rheumatoid arthritis, osteoarthritis, cancer (metastasis and invasion), liver disease such as hepatolienal fibrosis and cirrhosis, peridontitis, 5 pulmonary fibrosis, corneal ulcer, gastric ulcer, myocardiopathy, aneurysm, otosclerosis, epidermolysis bullosa, premature delivery, and atherosclerosis. On the other, its activity is suppressed in, for example, 10 diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis, herniated disk, etc.

Uses for the protein of the present invention or its partial peptide thereof, or a salt of either of them (hereinafter sometimes referred to briefly as the 15 protein of the present invention), the DNA coding for the protein or its partial peptide of the invention (hereinafter sometimes referred to briefly as the DNA of the present invention), the antibody against the protein of the present invention (hereinafter sometimes referred to as the antibody of the present invention), 20 and the antisense DNA are now described.

(1) Therapeutic or prophylactic composition for various diseases with which the protein of the present invention is associated

25 In the event of an abnormality or defect in the DNAs of metalloproteases, or when the expression or the activity of metalloproteases is suppressed, various diseases such as diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal 30 fibrosis, hepatocirrhosis, osteopetrosis and herniated disk are induced.

35 Therefore, the protein or the DNA of the present invention can be used as a pharmaceutical composition such as a therapeutic or prophylactic composition for a variety of diseases which associates with an abnormal expression or activity of metalloprotease such as

diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis, herniated disk, and so on.

For example, when there is a patient whose signal transductions in cells cannot function sufficiently or normally because of a decrease or a defect in the metalloproteases *in vivo*, the role of the metalloproteases for said patient can be expected sufficiently or normally by:

- 10 (a) administering the DNA coding for the protein of the present invention to the patient to express it;
- (b) inserting the DNA coding for the protein of the present invention into cells to express it and transplanting the cells to said patient, or
- 15 (c) administering the protein to the patient.

When the DNA of the present invention is used as the above-mentioned pharmaceutical composition, said DNA may be used alone or after inserting it into a suitable vector such as retrovirus vector, adenovirus vector, adenovirus-associated virus vector, pox virus etc. followed by subjecting the product vector to a conventional means. The DNA can also be administered as "naked" DNA, with physiologically acceptable carriers such as adjuvants to assist in uptake, by "gene" gun or by a catheter such as a catheter with a hydrogel.

If one wishes to use the protein of the present invention, one would use it in a purified form, preferably in a purity of at least 90%, more preferably at least 95%, still more preferably at least 98% and most preferably at least 99%.

For example, the protein of the present invention can be used orally in the form of tablets which may be sugar coated as necessary, capsules, elixirs, microcapsules etc., or non-orally in the form of injectable preparations such as aseptic solutions and

suspensions in water or other pharmaceutically acceptable liquids. These preparations can be produced by mixing the protein of the present invention with physiologically acceptable carriers, flavoring agents, 5 excipients, vehicles, antiseptics, stabilizers, binders etc. in unit dosage forms required for generally accepted manners of pharmaceutical preparation. Active ingredient contents in these preparations are set so that an appropriate dose within the specified range is 10 obtained.

Additives which can be mixed in tablets, capsules etc. include binders such as gelation, corn starch, tragacanth and gum arabic, excipients such as crystalline cellulose, swelling agents such as corn 15 starch, gelatin and alginic acid, lubricants such as magnesium stearate, sweetening agents such as sucrose, lactose and saccharin, and flavoring agents such as peppermint, akamono oil and cherry. When the unit dosage form is the capsule, the above-mentioned 20 materials may further incorporate liquid carriers such as oils and fats. Sterile compositions for injection can be formulated by ordinary methods of pharmaceutical preparation such as by dissolving or suspending active ingredients, naturally occurring vegetable oils such as 25 sesame oil and coconut oil, etc. in vehicles such as water for injection to create pharmaceutical compositions.

Aqueous liquids for injection include 30 physiological saline and isotonic solutions containing glucose and other auxiliary agents, e.g., D-sorbitol, D-mannitol and sodium chloride, and may be used in combination with appropriate dissolution aids such as alcohols, e.g., ethanol, polyalcohols, e.g., propylene 35 glycol and polyethylene glycol, nonionic surfactants, e.g., polysorbate 80TM and HCO-50 etc. Oily liquids include sesame oil and soybean oil, and may be used in

combination with dissolution aids such as benzyl benzoate and benzyl alcohol. Furthermore the above-mentioned materials may also be formulated with buffers, e.g., phosphate buffer and sodium acetate buffer; soothing agents, e.g., benzalkonium chloride, 5 procaine hydrochloride; stabilizers, e.g., human serum albumin, polyethylene glycol; preservatives, e.g., benzyl alcohol, phenol; antioxidants etc. The thus-prepared pharmaceutical composition such as an 10 injectable liquid is normally filled in an appropriate ampule.

The vector comprising the DNA of the present invention can be formulated as well as mentioned above, and usually can be used non-orally.

15 Because the thus-obtained preparation is safe and of low toxicity, it can be administered to humans or mammals (e.g., rat, mouse, guinea pig, rabbit, sheep, pig, bovine, horse, cat, dog, monkey, etc.).

20 The dose of the protein of the present invention may vary depending on subject disease, subject of administration, way of administration, and so on. When the protein of the present invention is used, for example, for treating diabetic nephropathy by oral administration, the dose of the protein of the present 25 invention is normally about 0.1 to 100mg, preferably 1.0 to 50mg, and more preferably 1.0 to 20mg per day for an adult (weighing 60 kg). When the protein of the present invention is used, for example, for treating herniated disk by non-oral administration, it is 30 advantageous to administer the protein of the present invention to the diseased part in the form of injectable preparation at a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg per administration by an 35 intravenous injection for an adult (weighing 60 kg), depending on subject of administration, subject disease

and so on. For other animal species, corresponding doses as converted per 60 kg weight can be administered.

(2) Screening of compounds as candidates which are medicinally useful against diseases

5 Any compounds or their salts which activate the function, for example a proteolytic activity, of the protein of the present invention can be used as a pharmaceutical composition such as a therapeutic or prophylactic composition for diseases such as diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis, and herniated disk. Therefore, the protein of the present invention is useful as a screening reagent for compounds or their salts, which activate the function 10 of the protein of the present invention.

15 On the other hand, any compounds or their salts which inhibit the function of the protein of the present invention can be used as a pharmaceutical composition such as a therapeutic or prophylactic composition for wound, rheumatoid arthritis, 20 osteoarthritis, cancer (metastasis and invasion), liver disease such as hepatolienal fibrosis and cirrhosis, peridontitis, pulmonary fibrosis, corneal ulcer, gastric ulcer, myocardiopathy, aneurysm, otosclerosis, 25 epidermolysis bullosa, premature delivery, atherosclerosis, septemia, multiple (disseminated) sclerosis, cachexia, hypercalcemia, leukemia, lymphoma, diabetes, autoimmune diseases such as systemic lupus erythematosus, asthma, immune diseases such as allergic 30 rhinitis and atopic dermatitis, generalized inflammatory reactions associated with trauma, burn or acute pancreatitis, ischemia-reperfusion syndrome, cardiovascular diseases such as myocardial infarction, congestive heart failure, etc., organ transplantation 35 and graft-vs.-host disease (GVHD). Therefore, the protein of the present invention is useful as a

screening reagent for those compounds or their salts which inhibit the function of the protein of the present invention.

The present invention, therefore, provides

- 5 (1) a method for screening for a compound which activates the function (e.g. a proteolytic activity) of the protein of the present invention or its partial peptide, or a salt of either of them (such compound will sometimes be referred to as activator), or a
- 10 compound which inhibits the function of the protein of the present invention or its partial peptide thereof, or a salt of either of them (such compound will sometimes be referred to as inhibitor) characterized in that the protein of the present invention or its
- 15 partial peptide, or a salt of either of them, is used as a screening reagent.

More particularly, the invention provides

- (2) a method for screening for the activator or inhibitor, characterized by comparing the results in cases of (i) a substrate is contacted with the protein of the present invention and (ii) a substrate and a test compound are contacted with the protein of the present invention.

More specifically, the above screening method is characterized by measuring and comparing the proteolytic activity of the protein of the present invention in cases of (i) and (ii).

The substrate may include any substances which may function as substrates for the protein of the present invention. Examples of the substrate are casein, azocasein, FITC-casein, radio(e.g. ¹⁴C, ³H, etc.)labeled casein, collagen, azocollagen, FITC-collagen, radio(¹⁴C, ³H, etc.)-labeled collagen, and oligopeptides having a (7-methoxycoumarin-4-yl)acetyl group in the N-terminal domain and an N³-(2,4-dinitrophenyl)-2,3-diaminopropionyl group at a position towards the C-

terminus by a few residues from the position where the first mentioned group is attached.

5 Examples of the test compound that can be used includes but is not limited to peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products, cell extracts, plant extracts, and animal tissue extracts. Such compounds may be novel substances or known substances.

10 For carrying the above screening method into practice, the protein of the present invention is first suspended in a suitable screening buffer to prepare a sample. The buffer may be any buffer that does not affect the binding of the protein of the present invention to the substrate, such as a phosphate buffer or Tris-HCl buffer in a pH range of about 4 to 10 15 (preferably pH about 6 to 8).

20 The proteolytic activity of the protein of the present invention can be determined by the known PER method (F. T. Lundy et al. Electrophoresis, 16, 43, 1995), and so on. Specifically, any test compounds 25 that activate the proteolytic activity by not less than about 20%, preferably not less than 30%, more preferably not less than 50%, in experiment (ii) as compared with experiment (i) can be selected as an activator of the proteolytic activity of the protein of the present invention, while any test compounds that inhibit the proteolytic activity by not less than about 20%, preferably not less than 30%, more preferably not less than 50%, in experiment (ii) as compared with 30 experiment (i) can be selected as an inhibitor of the proteolytic activity of the protein of the present invention.

35 The screening kit of the present invention comprises the protein of the present invention or peptide, or a salt of either of them. The following is a typical screening kit embodying the principle of the

present invention.

Screening reagents:

(1) Screening buffer

Tris-HCl buffer, pH 8.0

5 (sodium chloride and calcium chloride contained)

(2) Protein sample

The protein of the invention or its partial peptide

(3) Substrate

10 Casein 20 mg/ml

(4) Detection

Coomassie Brilliant Blue (CBB) staining

Assay protocol:

15 Add aminophenyl mercuric acetate (final concentration 1 mM) to the protein of the present invention and react at 37°C for 30 minutes. Electrophorese the reaction mixture on SDS-polyacrylamide gels (non-reducing) in accordance with PER (F. T. Lundy et al., Electrophoresis, 16, 43, 1995). Then, saturate the 20 polyacrylamide gels with the substrate and react in the reaction medium at 37°C for 16 hours. After the reaction, stain the gels with CBB to detect a proteolytic activity.

25 The compound or a salt thereof which can be identified by the screening method of the present invention or by using the screening kit of the present invention is a compound selected from among a peptide, protein, nonpeptide compound, synthetic compound, 30 fermentation product, cell extract, plant extract, or animal tissue extract, which activates or inhibits the function of the protein of the present invention.

The salt of the compound may be the same those as mentioned above as to the protein of the present 35 invention.

The compound which activates the function (e.g.

proteolytic activity) of the protein of the present invention is safe and of low toxicity, and can be used as therapeutic and prophylactic composition for various diseases such as diabetic nephropathy,
5 glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis, herniated disk and so on.

On the other hand, the compound which inhibits the function (e.g. proteolytic activity) of the protein of the present invention is safe and of low toxicity, and can be used as therapeutic and prophylactic composition for various diseases such as wound, rheumatoid arthritis, osteoarthritis, cancer (metastasis and invasion), liver disease such as hepatolienal fibrosis and cirrhosis, periodontitis, pulmonary fibrosis, corneal ulcer, gastric ulcer, myocardiopathy, aneurysm, otosclerosis, epidermolysis bullosa, premature delivery, atherosclerosis, septemia, multiple (disseminated) sclerosis, cachexia, hypercalcemia, 10 leukemia, lymphoma, diabetes, autoimmune diseases such as systemic lupus erythematosus, asthma, immune diseases such as allergic rhinitis and atopic dermatitis, generalized inflammatory reactions associated with trauma, burn or acute pancreatitis, 15 ischemia-reperfusion syndrome, cardio-vascular diseases such as myocardial infarction, congestive heart failure, etc., organ transplantation, graft-vs.-host disease (GVHD) and so on.

The compound which is identified by the screening method or the screening kit can be used as the above-mentioned therapeutic or prophylactic composition in accordance with a conventional means. The compound can be used in the form of tablets, capsules, elixirs, microcapsules, aseptic solutions, suspensions and so on 30 as well as the pharmaceutical composition comprising the protein of the present invention as mentioned 35

above.

Because the thus-obtained preparation is safe and of low toxicity, it can be administered to humans or mammals (e.g., rats, rabbits, sheep, pigs, bovines, 5 cats, dogs, monkeys, etc.).

The dose of the compound or a salt thereof may vary depending on subject disease, subject of administration, way of administration, and so on. When the compound inhibiting the function of the protein of 10 the present invention is used, for example, for treating diabetic nephropathy by oral administration, the dose of the compound is normally about 0.1 to 100mg, preferably about 1.0 to 50mg, and more preferably about 1.0 to 20mg per day for an adult (weighing 60 kg). When the compound inhibiting the 15 function of the protein of the present invention is used, for example, for treating diabetic nephropathy by non-oral administration, it is advantageous to administer the compound in the form of injectable preparation at a daily dose of about 0.01 to 30 mg, 20 preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg per administration by an intravenous injection for an adult (weighing 60 kg), depending on subject of administration, subject disease and so on. 25 For other animal species, corresponding does as converted per 60 kg weight can be administered.

(3) Quantitative determination of the protein of the present invention

The antibody of the present invention is capable 30 of specifically recognizing the protein of the present invention and, accordingly, it can be used for quantitative determination of the protein of the present invention in test liquid samples and particularly for quantitative determination by sandwich 35 immunoassays.

Thus, the present invention provides, for example,

the following methods:

(i) a quantitative determination of the protein of the present invention in a test liquid sample, which comprises

5 (a) competitively reacting the test liquid sample and a labeled protein of the present invention with the antibody of the present invention, and

(b) measuring the ratio of the labeled protein of the present invention binding with said antibody; and

10 (ii) a quantitative determination of the protein of the present invention in a test liquid sample, which comprises

(a) reacting the test liquid sample with an antibody immobilized on an insoluble carrier and a labeled

15 antibody simultaneously or continuously, and

(b) measuring the activity of the labeling agent on the insoluble carrier,

wherein one antibody is capable of recognizing the N-terminal region of the protein of the present invention

20 while another antibody is capable of recognizing the C-terminal region of the protein of the present invention.

When the monoclonal antibody of the present invention recognizing a protein of the present

25 invention (hereinafter, sometimes referred to as "monoclonal antibody of the present invention") is

used, the quantity of the protein of the present invention can be measured and, moreover, the protein of

30 the present invention can be detected by means of a tissue staining, etc. as well. For such an object, antibody molecules per se may be used, or $F(ab')_2$.

Fab' or Fab fractions of the antibody molecule may also be used. There is no particular limitation for the

35 measuring method using the antibody of the present invention and any measuring method may be used so far as it relates to a method in which the amount of

antibody, antigen or antibody-antigen complex, depending on or corresponding to the amount of antigen, e.g. the amount of the protein of the present invention in the liquid sample to be measured, is detected by a 5 chemical or a physical means and then calculated using a standard curve prepared by a standard solution containing the known amount of antigen. For example, nephrometry, competitive method, immunometric method and sandwich method are suitably used and, in terms of 10 sensitivity and specificity, the sandwich method which will be described herein later is particularly preferred.

Examples of the labeling agent used in the measuring method using the labeling substance are 15 radioisotopes, enzymes, fluorescent substances, luminescent substances, colloids, magnetic substances, etc.. Examples of the radioisotope are [¹²⁵I], [¹³¹I], [³H] and [¹⁴C]. Preferred examples of the enzyme are those which are stable and with much specific activity, 20 such as β -galactosidase, β -glucosidase, alkaline phosphatase, peroxidase and malate dehydrogenase. Examples of the fluorescent substance are fluorescamine, fluorescein isothiocyanate, etc.. Examples of the luminescent substance are luminol, 25 luminol derivatives, luciferin, lucigenin, etc.. Further, a biotin-avidin system may also be used for binding an antibody or antigen with a labeling agent.

In an insolubilization (immobilization) of antigens or antibodies, a physical adsorption may be 30 used or a chemical binding which is usually used for insolubilization or immobilization of proteins or enzymes may be used as well. Examples of the carrier are insoluble polysaccharides such as agarose, dextran and cellulose; synthetic resins such as polystyrene, 35 polyacrylamide and silicone; glass; etc..

In a sandwich method, the test liquid is made to

react with an insolubilized monoclonal antibody of the present invention (the first reaction), then it is allowed to react with an another labeled monoclonal antibody of the present invention (the second reaction) 5 and the activity of the labeling agent on the insoluble carrier is measured whereupon the amount of the protein of the present invention in the test liquid can be determined. The first reaction and the second reaction may be conducted reversely or simultaneously or they 10 may be conducted with an interval. The type of the labeling agent and the method of insolubilization may be the same as those mentioned hereinbefore. In the immunoassay by means of a sandwich method, it is not always necessary that the antibody used for the labeled 15 antibody and the antibody for the solid phase is one type or one species but, with an object of improving the measuring sensitivity, etc., a mixture of two or more antibodies may be used as well.

In the method of measuring the protein of the 20 present invention by the sandwich method of the present invention, the preferred monoclonal antibodies of the present invention used for the first and the second reactions are antibodies wherein their sites binding to the protein of the present invention are different from 25 each other. Thus, antibodies used in the first and the second reactions are those wherein, when an antibody used in the second reaction recognizes the C-terminal region of the protein of the present invention, then another antibody recognizing the site other than C-terminal regions, e.g. recognizing the N-terminal 30 region, is preferably used in the first reaction.

The monoclonal antibody of the present invention 35 may be used in a measuring system other than the sandwich method such as a competitive method, an immunometric method and a nephrometry. In the competitive method, an antigen in the test solution and

a labeled antigen are allowed to react with an antibody in a competitive manner, then an unreacted labeled antigen (F) and a labeled antigen (B) binding with an antibody are separated (i.e. B/F separation) and the 5 labeled amount of any of B and F is measured whereupon the amount of the antigen in the test solution is determined. With respect to a method for such a reaction, there are a liquid phase method in which a soluble antibody is used as the antibody and the B/F 10 separation is conducted by polyethylene glycol, a second antibody to the above-mentioned antibody, etc.; and a solid phase method in which an immobilized antibody is used as the first antibody or a soluble antibody is used as the first antibody while an 15 immobilized antibody is used as the second antibody.

In the immunometric method, an antigen in the test solution and an immobilized antigen are subjected to a competitive reaction with a certain amount of a labeled antibody followed by separating into solid and liquid 20 phases or the antigen in the test solution and an excess amount of labeled antibody are allowed to react, then an immobilized antigen is added to bind an unreacted labeled antibody with the solid phase and separated into solid and liquid phases. After that, 25 the labeled amount of any of the phases is measured to determine the antigen amount in the test solution.

In the nephrometry, the amount of insoluble sediment which is produced as a result of the antigen-antibody reaction in a gel or in a solution is 30 measured. Even when the antigen amount in the test solution is small and only a small amount of the sediment is obtained, a laser nephrometry wherein scattering of laser is utilized can be suitably used.

In applying each of those immunological measuring 35 methods (immunoassays) to the measuring method of the present invention, it is not necessary to set up any

special condition, operation, etc. therefor. A measuring system (assay system) for the protein of the present invention may be constructed taking the technical consideration of the persons skilled in the 5 art into consideration in the conventional conditions and operations for each of the methods. With details of those conventional technical means, a variety of reviews, reference books, etc. may be referred to.

They are, for example, Hiroshi Irie (ed):
10 "Radioimmunoassay" (Kodansha, Japan, 1974); Hiroshi Irie (ed): "Radioimmunoassay; Second Series" (Kodansha, Japan, 1979); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay" (Igaku Shoin, Japan, 1978); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay" (Second Edition) (Igaku Shoin, Japan, 1982); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay" (Third Edition) (Igaku Shoin, Japan, 1987); "Methods in Enzymology" Vol. 70 (Immunochemical Techniques (Part A)); ibid. Vo. 73 (Immunochemical Techniques (Part B)); ibid. Vo. 74 (Immunochemical Techniques (Part C)); ibid. Vo. 84 (Immunochemical Techniques (Part D: Selected Immunoassays)); ibid. Vol. 92 (Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)); ibid. Vol. 121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (Academic Press); etc.

By using the antibody of the present invention in the above manner, the protein of the present invention can be assayed with high sensitivity.

30 In addition, when increase in concentration of the protein of the present invention is detected by determining the concentration of the protein of the present invention by using the antibody against the protein of the present invention, it may lead, with 35 high probability, to the diagnosis of various diseases such as wound, rheumatoid arthritis, osteoarthritis,

cancer (metastasis and invasion), liver disease such as hepatolienal fibrosis and cirrhosis, peridontitis, pulmonary fibrosis, corneal ulcer, gastric ulcer, myocardiopathy, aneurysm, otosclerosis, epidermolysis bullosa, premature delivery, atherosclerosis, septemia, multiple (disseminated) sclerosis, cachexia, hypercalcemia, leukemia, lymphoma, diabetes, autoimmune diseases such as systemic lupus erythematosus, asthma, immune diseases such as allergic rhinitis and atopic dermatitis, generalized inflammatory reactions associated with trauma, burn or acute pancreatitis, ischemia-reperfusion syndrome, cardiovascular diseases such as myocardial infarction, congestive heart failure, etc., organ transplantation, graft-vs.-host disease (GVHD) and so on. When decrease in concentration of the protein of the present invention is detected, it may lead, with high probability, to the diagnosis of various diseases such as diabetic nephropathy, glomerulonephritis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis, herniated disk and so on.

Thus, the antibody of the present invention is useful as a diagnostic agent for the above-mentioned diseases.

Furthermore, the antibody of the present invention can be used for the purpose of detecting the protein of the present invention which may be present in test samples such as body fluids or tissues. The antibody can also be used for the construction of an antibody column for purification of the protein of the present invention, detection of the protein of the present invention in the fractions in the course of purification, and analysis of the behavior of the protein of the present invention in the test cell.

35 (4) Gene diagnostic agent

By using the DNA of the present invention as a

probe, for instance, an abnormality (gene abnormality) of the DNA or mRNA coding for the protein of the present invention or its partial peptide in humans or mammals (e.g. rat, mouse, guinea pig, rabbit, sheep, swine, 5 bovine, horse, cat, dog, monkey, chimpanzee, etc.) can be detected. Therefore, the DNA of the present invention is useful as a gene diagnostic agent for the damage to the DNA or mRNA, mutation thereof, or decreased expression thereof, or increased expression or overexpression of the 10 DNA or mRNA.

The above-mentioned gene diagnosis using the DNA of the present invention can be carried out by, for example, the per se known Northern hybridization assay or PCR-SSCP assay [Genomics, 5, 874-879 (1989); Proceedings of the 15 National Academy of Sciences of the United States of America, 86, 2766-2770 (1989)].

When increase in expression of the DNA is detected by Northern hybridization assay, it may lead, with high probability, to the diagnosis of wound, rheumatoid 20 arthritis, osteoarthritis, cancer (metastasis and invasion), liver disease such as hepatolienal fibrosis and cirrhosis, periodontitis, pulmonary fibrosis, corneal ulcer, gastric ulcer, myocardiopathy, aneurysm, otosclerosis, epidermolysis bullosa, premature delivery, 25 atherosclerosis, septemia, multiple (disseminated) sclerosis, cachexia, hypercalcemia, leukemia, lymphoma, diabetes, autoimmune diseases such as systemic lupus erythematosus, asthma, immune diseases such as allergic rhinitis and atopic dermatitis, generalized inflammatory 30 reactions associated with trauma, burn or acute pancreatitis, ischemia-reperfusion syndrome, cardiovascular diseases such as myocardial infarction, congestive heart failure, etc., organ transplantation, graft-vs.-host disease (GVHD) and so on. When decrease 35 in expression of the DNA is detected, it may lead, with high probability, to the diagnosis of diabetic

nephropathy, glomerulonephritis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis, herniated disk and so on. When a mutation of the DNA is detected by the PCR-SSCP assay, it may lead, with high probability to

5 diagnosis of wound, rheumatoid arthritis, osteoarthritis, cancer (metastasis and invasion), liver disease such as hepatolienal fibrosis and cirrhosis, periodontitis, pulmonary fibrosis, corneal ulcer, gastric ulcer, myocardiopathy, aneurysm, otosclerosis,

10 epidermolysis bullosa, premature delivery, atherosclerosis, septemia, multiple (disseminated) sclerosis, cachexia, hypercalcemia, leukemia, lymphoma, diabetes, autoimmune diseases such as systemic lupus erythematosus, asthma, immune diseases such as allergic rhinitis and atopic dermatitis, generalized inflammatory reactions associated with trauma, burn or acute pancreatitis, ischemia-reperfusion syndrome, cardiovascular diseases such as myocardial infarction, congestive heart failure, etc., organ transplantation,

15 graft-vs.-host disease (GVHD), diabetic nephropathy, glomerulonephritis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis, herniated disk and so on.

20

(5) Antisense DNA

25 An antisense DNA capable of making complementary conjugate with the DNA of the present invention to suppress expression of the DNA is capable of inhibiting the function of the protein or the DNA of the present invention in the body. Therefore, the antisense DNA can be used as a pharmaceutical composition such as a therapeutic and prophylactic composition for diseases such as wound, rheumatoid arthritis, osteoarthritis, cancer (metastasis and invasion), liver disease such as hepatolienal fibrosis and cirrhosis, periodontitis,

30 pulmonary fibrosis, corneal ulcer, gastric ulcer, myocardiopathy, aneurysm, otosclerosis, epidermolysis

35

5 bullosa, premature delivery, atherosclerosis, septemia, multiple (disseminated) sclerosis, cachexia, hypercalcemia, leulemia, lymphoma, diabetes, systemic lupus erythematosus, asthma, allergic rhinitis, atopic dermatitis, trauma, burn, acute pancreatitis, ischemia-reperfusion syndrome, myocardial infarction, congestive heart failure, organ transplantation, graft-vs.-host disease (GVHD) and so on.

10 The antisense DNA can be used as the above-mentioned pharmaceutical composition in the same manner as the pharmaceutical composition comprising the DNA of the present invention as mentioned above.

15 (6) Preparation of non-human animals harboring a foreign DNA coding for the protein of the present invention

20 Transgenic non-human animals which express the protein of the present invention can be constructed by using the DNA of the present invention. As the species of non-human animals that can be used, a variety of mammals (e.g. rat, mouse, rabbit, sheep, swine, bovine, cat, dog, monkey, etc.), etc. (hereafter referred to as animals) can be mentioned. Particularly preferred are mouse and rabbit.

25 In transferring the DNA of the present invention to a host animal, it is generally advantageous to use the DNA as a gene construct prepared by ligating the DNA downstream of a promoter capable of expressing the DNA in animal cells. For the transfer of a rabbit-derived DNA of the invention, for instance, a DNA 30 transgenic animal for high production of the protein of the present invention can be constructed by the microinjection of, for example, the fertilized rabbit egg with a gene construct prepared by ligating the DNA of the present invention as derived from an animal 35 having high homology therewith downstream of a promoter capable of causing the expression of the DNA of the

present invention in animal cells. As for such promoters, viral promoters or ubiquitous expression promoters such as and metallothionein promoters can also be used.

5 The transfer of the DNA into the fertilized egg cell stage is secured in all the germ and somatic cells of the host animal. The presence of the protein of the present invention in the germ cells of the DNA-transferred animal means that all the progeny of the 10 transgenic animal invariably harbors the protein of the present invention in their germ and somatic cells. The offsprings of such an animal to which the gene has been passed on have the protein of the present invention in all of their germ and somatic cells.

15 The transgenic animal in which the DNA of the present invention has been expressed is confirmed to retain the gene stably by copulation and then can be bred from generation to generation as the DNA-harboring animals in the usual breeding environment.

20 Furthermore, by mating male and female animals harboring the objective DNA, it is possible to obtain homozygotes having the introduced gene in both of the homologous chromosomes, and by mating such partners, it is possible to insure that all the progeny animals will 25 harbor this DNA.

25 The animal to which the DNA of the present invention has been passed on has the protein of the present invention expressed in a high degree so that it is useful as an animal for screening for compounds and 30 salts which would activate or inhibit the proteolytic activity of the protein of the present invention.

30 The animal to which the DNA of the present invention has been transferred can also be used as a source of cells for tissue culture. For example, the 35 protein of the present invention can be studied either directly by analyzing the DNA or RNA in the tissues of

a mouse to which the DNA of the present invention has been transferred or analyzing a tissue containing the protein of the present invention as expressed by the gene. It is possible to culture cells from a tissue 5 containing the protein of the present invention by the standard tissue culture technique and, using the culture, study the functions of cells derived from even those tissues which are generally difficult to culture, such as brain or peripheral tissue cells. Furthermore, 10 by using such cells, drugs which activate the functions of various tissues may be selected. Moreover, if a high-expression cell line is provided, it will be possible to isolate and purify the protein of the present invention from the cell line.

15 In the specification and drawings of the present application, the abbreviations used for bases (nucleotides), amino acids and so forth are those recommended by the IUPAC-IUB Commission on Biochemical 20 Nomenclature or those conventionally used in the art. Examples thereof are given below. Amino acids for which optical isomerism is possible are, unless otherwise specified, in the L form.

DNA : Deoxyribonucleic acid

25 CDNA : Complementary deoxyribonucleic acid

A : Adenine

T : Thymine

G : Guanine

C : Cytosine

30 RNA : Ribonucleic acid

mRNA : Messenger ribonucleic acid

dATP : Deoxyadenosine triphosphate

dTTP : Deoxythymidine triphosphate

dGTP : Deoxyguanosine triphosphate

35 dCTP : Deoxycytidine triphosphate

ATP : Adenosine triphosphate

EDTA : Ethylenediaminetetracetic acid
SDS : Sodium dodecyl sulfate
Gly : Glycine
Ala : Alanine
5 Val : Valine
Leu : Leucine
Ile : Isoleucine
Ser : Serine
Thr : Threonine
10 Cys : Cysteine
Met : Methionine
Glu : Glutamic acid
Asp : Aspartic acid
Lys : Lysine
15 Arg : Arginine
His : Histidine
Phe : Phenylalanine
Tyr : Tyrosine
Trp : Tryptophan
20 Pro : Proline
Asn : Asparagine
Gln : Glutamine
pGlu : Pyroglutamic acid
Me : Methyl
25 Et : Ethyl
Bu : Butyl
Ph : Phenyl
TC : Thiazolidine-4(R)-carboxamide
Substitution groups, protecting groups and
30 reagents used in the specification of the present
application are represented by the symbols set forth
below.
Tos : p-toluene sulfonyl
CHO : Formyl
35 Bzl : Benzyl
Cl₂-Bzl: 2,6-dichlorobenzyl

Bom : Benzyloxymethyl
Z : Benzyloxycarbonyl
Cl-Z : 2-chlorobenzyloxycarbonyl
Br-Z : 2-bromobenzyloxycarbonyl
5 Boc : Tert-butoxycarbonyl
DNP : Dinitrophenyl
Trt : Trityl
Bum : Tert-butoxymethyl
Fmoc : N-9-fluorenylmethyloxycarbonyl
10 HOEt : 1-hydroxybenzotriazole
HOOBT : 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
HONB : 1-hydroxy-5-norbornene-2,3-dicarboximide
DCC : Dicyclohexylcarbodiimide
Cha : Cyclohexyl alanyl
15 Abu : Aminobutyrate
Abz : 2-aminobenzoyl
Each SEQ ID NO set forth in the SEQUENCE LISTING
of the specification refers to the following sequence:
SEQ ID NO:1 shows an amino acid sequence of the
20 human liver-derived metalloprotease of the present
invention (Fig. 1).
SEQ ID NO:2 shows an amino acid sequence of the
rat liver-derived metalloprotease of the present
invention (Fig. 6).
25 SEQ ID NO:3 shows an amino acid sequence of the
partial peptide of the human liver-derived
metalloprotease of the present invention (Fig. 2),
wherein 97 amino acid residues are deleted from N-
terminus of the amino acid sequence represented by SEQ
30 ID NO:1.
SEQ ID NO:4 shows an amino acid sequence of the
partial peptide of the human liver-derived
metalloprotease of the present invention (Fig. 3),
wherein 92 amino acid residues are deleted from N-
35 terminus of the amino acid sequence represented by SEQ
ID NO:1.

SEQ ID NO:5 shows an amino acid sequence of the partial peptide of the rat liver-derived metalloprotease of the present invention, wherein 98 amino acid residues are deleted from N-terminus of the 5 amino acid sequence represented by SEQ ID NO:2.

SEQ ID NO:6 shows an amino acid sequence of the partial peptide of the rat liver-derived metalloprotease of the present invention, wherein 93 amino acid residues are deleted from N-terminus of the 10 amino acid sequence represented by SEQ ID NO:2.

SEQ ID NO:7 shows a nucleotide sequence of the DNA coding for the human liver-derived metalloprotease comprising an amino acid sequence represented by SEQ ID NO:1 of the present invention.

15 SEQ ID NO:8 shows a nucleotide sequence of the DNA coding for the rat liver-derived metalloprotease comprising an amino acid sequence represented by SEQ ID NO:2 of the present invention.

20 SEQ ID NO:9 shows a nucleotide sequence of the DNA coding for the partial peptide comprising an amino acid sequence represented by SEQ ID NO:3 of the human liver-derived metalloprotease of the present invention.

25 SEQ ID NO:10 shows a nucleotide sequence of the DNA coding for the partial peptide comprising an amino acid sequence represented by SEQ ID NO:4 of the human liver-derived metalloprotease of the present invention.

30 SEQ ID NO:11 shows a nucleotide sequence of the DNA coding for the partial peptide comprising an amino acid sequence represented by SEQ ID NO:5 of the human liver-derived metalloprotease of the present invention.

35 SEQ ID NO:12 shows a nucleotide sequence of the DNA coding for the partial peptide comprising an amino acid sequence represented by SEQ ID NO:6 of the human liver-derived metalloprotease of the present invention.

SEQ ID NO:13 shows a nucleotide sequence of the synthetic primer used for the cloning of the DNA coding

for the human liver-derived protein of the present invention in Example 1.

SEQ ID NO:14 shows a nucleotide sequence of the synthetic primer used for the cloning of the DNA coding for the rat liver-derived protein of the present invention in Example 1.

SEQ ID NO:15 shows a nucleotide sequence of the synthetic primer used for the construction of Escherichia coli expression vector in Example 4.

SEQ ID NO:16 shows a nucleotide sequence of the synthetic primer used for the construction of Escherichia coli expression vector in Example 4.

SEQ ID NO:17 shows a nucleotide sequence of the synthetic primer used for the cloning of the DNA coding for the rat liver-derived protein of the present invention in Example 9.

SEQ ID NO:18 shows a nucleotide sequence of the synthetic primer used for the cloning of the DNA coding for the rat liver-derived protein of the present invention in Example 9.

SEQ ID NO:19 shows a nucleotide sequence of the synthetic primer used for the cloning of the DNA coding for the rat liver-derived protein of the present invention in Example 9.

The transformant strain of Escherichia coli, designated DH10B/PTB1921, which is obtained in the Example 1 mentioned hereinafter, is on deposit under the terms of the Budapest Treaty from April 22, 1996, with the NIBH under the Accession Number of FERM BP-5516. It is also on deposit from April 19, 1996 with the IFO under the Accession Number of IFO 15950.

The transformant strain of Escherichia coli, designated DH10B/PTB1982, which is obtained in the Example 9 mentioned hereinafter, is on deposit under the terms of the Budapest Treaty from April 9, 1997, with the NIBH under the Accession Number of FERM BP-

5906. It is also on deposit from April 9, 1997 with the IFO under the Accession Number of IFO 16074.

Brief Description of Drawings

5 Figure 1 shows an amino acid sequence of a human liver-derived metalloprotease of the present invention.

10 Figure 2 shows an amino acid sequence of a partial peptide of the human liver-derived metalloprotease of the present invention, wherein 97 amino acid residues are deleted from the N-terminus of the amino acid sequence shown in Figure 1.

15 Figure 3 shows an amino acid sequence of a partial peptide of a human liver-derived metalloprotease of the present invention, wherein 92 amino acid residues are deleted from the N-terminus of the amino acid sequence shown in Figure 1.

Figure 4 shows a nucleotide sequence of a DNA encoding a human liver-derived metalloprotease of the present invention.

20 Figure 5 shows an electrophoretogram of Western blot analysis by using a human liver-derived metalloprotease of the present invention and the antibody against the protein. The abscissa represents a number of each samples. No. 1 shows a molecular weight marker used, No. 2 shows a culture supernatant of non-infected HighFive cells, No. 3 shows a culture supernatant of HighFive cells infected with β -galactosidase-expressing recombinant virus (Invitrogen), and No. 4 shows a culture supernatant of 25 HighFive cells infected with human liver-derived metalloprotease-expressing recombinant virus. The ordinate represents a distance of electrophoretic migration (cm).

30 Figure 6 shows an amino acid sequence of a rat liver-derived metalloprotease of the present invention and a nucleotide sequence of a DNA containing a DNA

coding for the amino acid sequence.

Best Mode for Carrying Out the Invention

Examples:

5 The following examples are intended to illustrate the present invention in further detail and should by no means be construed as defining the scope of the invention. Incidentally, the gene manipulations using Escherichia coli were made according to the protocol
10 described in Molecular Cloning.

Example 1

Cloning of a gene coding for human liver-derived metalloprotease

15 The cloning of the cDNA was carried out using Gene Trapper Positive Selection System (GIBCO/BRL).

16 Escherichia coli DH12S from a human liver-derived cDNA library (GIBCO/BRL) was cultured in Terrific Broth (12 g/l bacto-tryptone (Difco), 24 g/l bacto-yeast extract (Difco), 2.3 g/l potassium dihydrogen phosphate, 12.5 g/l potassium monohydrogen phosphate) at 30°C for 16 hours and using Qiagen Plasmid Kit (Qiagen), a plasmid cDNA library was purified and extracted. The purified plasmid cDNA library was
20 digested with Gene II and Exo III (both from GIBCO/BRL) to construct a single-stranded cDNA library.

21 On the other hand, as a probe, a synthetic oligonucleotide (SEQ ID NO:13) was used for the screening of the cDNA library. The probe was labeled by biotinylating its 3'-end with TdT and biotin-14-dCTP (GIBCO/BRL). The single-stranded cDNA library was treated at 95°C for 1 minute and, then, quenched on ice. To this was added the biotinylated probe, and hybridization was conducted at 37°C for 1 hour and at room temperature. After hybridization, Gene Trapper Positive Selection System magnet beads (GIBCO/BRL) were

added and the mixture was allowed to stand at room temperature for 30 minutes with stirring at 2-min intervals. Thereafter, the mixture was put in Gene Trapper Positive Selection System magnet track (GIBCO/BRL) and allowed to stand for 2 minutes. The supernatant was then discarded and the magnet beads were washed with Gene Trapper Positive Selection System wash buffer. This washing with the wash buffer was repeated 3 times. The beads were then placed and allowed to sit in the magnetic track and the supernatant was discarded. Then, Gene Trapper Positive Selection System elution buffer was added and the system was allowed to stand at room temperature for 5 minutes. The system was put in the magnetic track and left standing for 5 minutes, and the supernatant DNA solution was recovered.

The synthetic oligonucleotide (SEQ ID NO:13) as the primer was put in the recovered DNA solution and the system was allowed to stand at 95°C for 1 minute. Then, Gene Trapper Positive Selection System repair enzyme was added and the mixture was allowed to stand at 70°C for 15 minutes to synthesize a double-stranded DNA. Using an electroporation apparatus (Bio-Rad), this synthetic double-stranded DNA was introduced into Escherichia coli DH10B.

Using the resulting transformants and, as primers, 2 oligonucleotides (SEQ ID NO:13 and NO:14), a screening by colony PCR was carried out. A colony line of amplified fragments of 510 bp formed by PCR was selected as positive clones.

The selected Escherichia coli was cultured and the DNA was extracted. The reaction was carried out using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase FS (Perkin-Elmer) and the nucleotide sequence of the cDNA fragment was determined with 377 DNA Sequencer (Perkin-Elmer).

The clone obtained was found to have the 2264bp containing the poly(A)⁺ chain and a sequence of 1524bp as represented by SEQ ID NO:7. Encoded in this cDNA fragment was a novel metalloprotease consisting of 508 5 amino acid residues as represented by SEQ ID NO:1 and the active center histidine residue had also been conserved. The homology with the known human metalloproteases (e.g. MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-17, MT- 10 MMP-1, MT-MMP-2, MT-MMP-3) at the amino acid level was as low as 30 to 36%.

The plasmid pTB1921 harboring the DNA encoding the human liver-derived metalloprotease of the invention was introduced into Escherichia coli DH10B to obtain 15 the transformant Escherichia coli DH10B/pTB1921.

Example 2

Transient expression of the human liver-derived metalloprotease and preparation of a culture 20 supernatant

The pTB1921 obtained in Example 1, which had been inserted into the expression plasmid pCMV-SPORT (GIBCO/BRL), was used for expression in animal cells. The COS-7 cell line (purchased from the Institute for 25 Fermentation, Osaka) was cultured in serum-containing DMEM and subcultured on the day before introduction of the gene. When the culture became 50% confluent, the COS-7 cells were washed with serum-free DMEM, and 2.5 ml of serum-free medium was added. To this cell 30 suspension was added TRANSFECTAM (Nippon Gene) containing 5 µg pTB1921, and the mixture was incubated under 5% CO₂ at 37°C for 4 hours. Then, 20% bovine serum-containing DMEM (2.5 ml) was added and the mixture was further incubated for 20 hours. The medium 35 was then replaced with serum-free DMEM and the culture supernatant was recovered 3 days later. As a control,

a mock culture supernatant obtained by adding TRANSFECTAM alone in otherwise the same manner was used.

5 **Example 3**

PER assay of the metalloprotease activity of the human liver-derived metalloprotease

To the cell culture supernatant obtained in Example 2 was added aminophenyl-mercuric acetate (final concentration 1 mM) and the reaction was carried out at 37°C for 30 minutes. The activity was then determined by the PER assay (F. T. Lundy et al., Electrophoresis, 16, 43, 1995). As a result, casein hydrolyzing activity which was not found in the mock culture supernatant was detected in the culture supernatant of COS-7 cells transfected with pTB1921. It was found that this activity was inhibited by o-phenanthroline.

20 **Example 4**

Construction of the *Esherichia coli* expression vector

To generate SphI and PstI cutting sites to cDNA encoding the human liver-derived metalloprotease of the present invention, using the pTB1921 obtained in Example 1 as the template and the following two oligonucleotides

25 5'-CCCGCATGCTACCTGTTGCTGGGCCGCTG-3' (SEQ ID NO:15)
5'-AAGCTGCAGATCTACGGTCTGGCGCCTGCTACA-3' (SEQ ID NO:16)
as primers, PCR (94°C x 30 sec., 55°C x 30 sec, 72°C x 1 min., 25 cycles) was carried out in accordance with 30 the protocol accompanying the PCR amplification kit (Takara Shuzo). After the PCR product was purified by using SpinBind PCR Purification System (FMC), it was subcloned into pCRII (Invitrogen). After confirmation of being free from error of the nucleotide sequence of 35 metalloprotease cDNA, the cDNA was cut out with SphI and PstI and ligated to similarly treated pQE30

(Qiagen). Then, Escherichia coli JM109 (Takara Shuzo) was transfected using the ligation mixture to obtain the human liver-derived metalloprotease-expressing Escherichia coli JM109/pNHMB.

5

Example 5

Expression of the recombinant metalloprotease in Escherichia coli and its purification

Using the Escherichia coli JM109/pNHMB as obtained in Example 4, a recombinant metalloprotease was obtained. Its expression in Escherichia coli and purification were carried out in accordance with the protocol accompanying QIAexpress System (Qiagen). As a result, the objective metalloprotease of about 46 kDa was eluted with buffer E (QIAexpress System). Then, using a dialysis membrane with a cutoff molecular weight of 12000-14000 (SPECTORAPOR), the eluate was dialyzed against buffer (0.2 M Tris-HCl (pH 9.0), 3 mM 2-mercaptoethanol, 0.3 mM 2-hydroxyethyl disulfide, 2 M urea, 0.1% Triton X-100) at 4°C for 16 hours and, then, serially against a buffer (0.05 M Tris-HCl (pH 8.0), 0.15 M NaCl, 0.1 M urea, 1 mM 2-mercaptoethanol, 0.1 mM 2-hydroxyethyl disulfide, 0.05% Triton X-100) and another buffer (0.05 M Tris-HCl (pH 8.0), 0.15 M NaCl, 0.05% Triton X-100) at 4°C for 4 hours each. In this manner, 18.2 mg of a recombinant human liver-derived metalloprotease could be obtained from 800 ml of the Escherichia coli culture.

30

Example 6

Establishment of an inhibitor screening system

A 96-well plate (Fluoro B Plate, Dainippon Pharmaceutical) was filled in with 30 µL of buffer (0.25 M Tris-HCl (pH 8.0), 5 mM CaCl₂, 100 mM NaCl, 10 µM ZnCl₂) and 20 µL of the recombinant human liver-derived metalloprotease (2.4 mg/ml) obtained in Example 5.

After 10 minutes of preincubation at 37°C, the enzymatic reaction was initiated by adding 100 µL of 10 µM substrate [DNP-Pro-Cha-Abu-Cys(Me)-His-Ala-Lys(N-Me-Abz)-NH₂; Bachem]. The reaction was conducted at 37°C for 16 hours and, using a microplate reader (MTP-32, Corona Electronic), the intensity of fluorescence in the reaction system was measured at an exciting wavelength of 365 nm and an emission wavelength of 460 nm. Compared with the intensity of fluorescence of enzyme-free control (-20), a buffer containing the renatured recombinant metalloprotease showed a fluorescence intensity of 230. When actinonin (Peptide Institute), a metalloprotease inhibitor, was added at a varying concentration to this reaction mixture, it inhibited metalloprotease activity with a 50% inhibitory concentration of about 10 µM. It was, therefore, clear that this assay system can be used for the screening of inhibitors of the human liver-derived metalloprotease.

20 Example 7

Acquisition of anti-metalloprotease polyclonal antibody

The recombinant human liver-derived metalloprotease (200 µg) obtained in Example 5 was suspended in complete Freund's adjuvant and injected into a Japanese white rabbit for the first immunization dose. Then, the rabbit was boosted with a suspension of 400 µg recombinant human liver-derived metalloprotease in incomplete Freund's adjuvant 4 times at 2-week intervals. Total blood was harvested at one week after the last booster administration to obtain about 50 ml of serum.

The antibody titer was determined as follows. To a 96-well plate prepared by immobilizing 0.5 µg/well of recombinant metalloprotease and blocked with BSA, diluted rabbit serum was added and the plate was allowed to stand at room temperature for 2 hours.

After washing with the PBS containing 0.1% Tween-20, anti-rabbit IgG-peroxidase (Capel) was added and the plate was allowed to stand for 2 hours. After washing, citrate-phosphate buffer containing o-phenylenediamine and hydrogen peroxide was added and a colorization reaction was carried out for 20 minutes. The reaction was stopped with 1 M sulfuric acid and using a plate reader, the colorization was read at 492 nm. As a result, an antiserum showing an antibody titer about 10 1×10^4 -fold higher than that of a non-immunized rabbit was obtained.

Example 8

15 Expression of the human liver-derived metalloprotease in insect cells and Western blotting with anti-metalloprotease antibody

Construction of a recombinant vaculovirus and expression in insect cells were carried out using Invitrogen's Bac-N-Blue Transfection Kit according to 20 the accompanying protocol. The plasmid obtained by introducing metalloprotease cDNA into the SacI and PstI restriction enzyme cleavage site of the expression vector pBlueBac4 (Invitrogen) and Bac-N-Blue (Invitrogen) viral DNA were introduced into Spodoptera frugiperda (Sf-9) cells, in which recombination was 25 allowed to take place. From the blue plaques, a recombinant virus containing the human liver-derived metalloprotease cDNA was picked and used to infect HighFive insect cells (Invitrogen). The culture supernatant of the above insect cells was Western-blotted 30 with the anti-metalloprotease antibody obtained in Example 7. As the secondary antibody, anti-rabbit IgG-alkaline phosphatase conjugate was used, and as color reagents, 5-bromo-4-chloro-3-indolyl-1-phosphate and 35 Nitro Blue Tetrazolium (both from Promega) were used. As shown in Fig. 5, a band of about 44 kb was observed

only in the culture supernatant of the HighFive cells infected with metalloprotease-expressed recombinant virus. The above results indicated that the antibody prepared in Example 7 could recognize the human liver-derived metalloprotease of the present invention.

Example 9

Cloning of a gene coding for rat liver-derived metalloprotease.

10 Synthetic oligonucleotide primer:

5'-GGCAGGGATCCAGGCTCTC-3' (SEQ ID NO:17)

5'-TGCATCCAGGTTAGGTTC-3' (SEQ ID NO:18)

15 prepared based on the nucleotide sequence of the cDNA coding for the human liver-derived metalloprotease obtained in Example 1 were used for PCR with rat brain and liver cDNA libraries (GIBCO/BRL) as templates. As a result, about 400 bp fragment coding for a part of the rat liver-derived metalloprotease was amplified from both rat cDNA libraries. The DNA fragment from 20 the brain cDNA library was subcloned into pCRII

(Invitrogen) and sequenced as described in Example 1.

Full-length cDNA coding for the rat liver-derived

metalloprotease was obtained from the rat liver cDNA library with the synthetic oligonucleotide primer:

25 5'-GCCGGAGCCAGAAGATGAGG-3' (SEQ ID NO:19)

prepared based on the 400bp fragment according to the method as described in the Example 1.

The cDNA consisted of 2,049bp and contained 1,551bp of open reading frame coding for 517 amino acids of rat liver-derived metalloprotease and poly(A)' as shown in Fig. 6. The rat liver-derived metalloprotease is 80% identical to the human liver-derived metalloprotease at the amino acid level.

35 Escherichia coli DH10B was transformed with the plasmid pTB1982 comprising the cDNA coding for the rat liver-derived metalloprotease to obtain the transformant:

Escherichia coli DH10B/pTB1982.

Industrial Applicability

The DNA coding for the protein of the present invention can be used as a therapeutic and prophylactic composition for a variety of diseases including diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis and herniated disk. The protein of the present invention is useful as a screening reagent for any compounds which activate or inhibit the function of the protein of the present invention. In addition, the antibody against the protein of the present invention specifically recognizes the protein of the present invention and can be used in the quantitative determination of the protein of the present invention in a test fluid.

SEQUENCE LISTING

INFORMATION FOR SEQ ID NO:1

(i) SEQUENCE CHARACTERISTICS

5 (A) LENGTH: 508

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1

10

Met Asn Cys Gln Gln Leu Trp Leu Gly Phe Leu Leu Pro Met Thr Val

1 5 10 15

Ser Gly Arg Val Leu Gly Leu Ala Glu Val Ala Pro Val Asp Tyr Leu

20 25 30

15 Ser Gln Tyr Gly Tyr Leu Gln Lys Pro Leu Glu Gly Ser Asn Asn Phe

35 40 45

Lys Pro Glu Asp Ile Thr Glu Ala Leu Arg Ala Phe Gln Glu Ala Ser

50 55 60

Glu Leu Pro Val Ser Gly Gln Leu Asp Asp Ala Thr Arg Ala Arg Met

20 65 70 75 80

Arg Gln Pro Arg Cys Gly Leu Glu Asp Pro Phe Asn Gln Lys Thr Leu

85 90 95

Lys Tyr Leu Leu Leu Gly Arg Trp Arg Lys Lys His Leu Thr Phe Arg

100 105 110

25 Ile Leu Asn Leu Pro Ser Thr Leu Pro Pro His Thr Ala Arg Ala Ala

115 120 125

Leu Arg Gln Ala Phe Gln Asp Trp Ser Asn Val Ala Pro Leu Thr Phe

130 135 140

Gln Glu Val Gln Ala Gly Ala Ala Asp Ile Arg Leu Ser Phe His Gly

30 145 150 155 160

Arg Gln Ser Ser Tyr Cys Ser Asn Thr Phe Asp Gly Pro Gly Arg Val

165 170 175

Leu Ala His Ala Asp Ile Pro Glu Leu Gly Ser Val His Phe Asp Glu

180 185 190

35 Asp Glu Phe Trp Thr Glu Gly Thr Tyr Arg Gly Val Asn Leu Arg Ile

195 200 205

Ile Ala Ala His Glu Val Gly His Ala Leu Gly Leu Gly His Ser Arg
 210 215 220
 Tyr Ser Gln Ala Leu Met Ala Pro Val Tyr Glu Gly Tyr Arg Pro His
 225 230 235 240
 5 Phe Lys Leu His Pro Asp Asp Val Ala Gly Ile Gln Ala Leu Tyr Gly
 245 250 255
 Lys Lys Ser Pro Val Ile Arg Asp Glu Glu Glu Glu Thr Glu Leu
 260 265 270
 Pro Thr Val Pro Pro Val Pro Thr Glu Pro Ser Pro Met Pro Asp Pro
 10 275 280 285
 Cys Ser Ser Glu Leu Asp Ala Met Met Leu Gly Pro Arg Gly Lys Thr
 290 295 300
 Tyr Ala Phe Lys Gly Asp Tyr Val Trp Thr Val Ser Asp Ser Gly Pro
 305 310 315 320
 15 Gly Pro Leu Phe Arg Val Ser Ala Leu Trp Glu Gly Leu Pro Gly Asn
 325 330 335
 Leu Asp Ala Ala Val Tyr Ser Pro Arg Thr Gln Trp Ile His Phe Phe
 340 345 350
 Lys Gly Asp Lys Val Trp Arg Tyr Ile Asn Phe Lys Met Ser Pro Gly
 20 355 360 365
 Phe Pro Lys Lys Leu Asn Arg Val Glu Pro Asn Leu Asp Ala Ala Leu
 370 375 380
 Tyr Trp Pro Leu Asn Gln Lys Val Phe Leu Phe Lys Gly Ser Gly Tyr
 385 390 395 400
 25 Trp Gln Trp Asp Glu Leu Ala Arg Thr Asp Phe Ser Ser Tyr Pro Lys
 405 410 415
 Pro Ile Lys Gly Leu Phe Thr Gly Val Pro Asn Gln Pro Ser Ala Ala
 420 425 430
 Met Ser Trp Gln Asp Gly Arg Val Tyr Phe Phe Lys Gly Lys Val Tyr
 30 435 440 445
 Trp Arg Leu Asn Gln Gln Leu Arg Val Glu Lys Gly Tyr Pro Arg Asn
 450 455 460
 Ile Ser His Asn Trp Met His Cys Arg Pro Arg Thr Ile Asp Thr Thr
 465 470 475 480
 35 Pro Ser Gly Gly Asn Thr Thr Pro Ser Gly Thr Gly Ile Thr Leu Asp
 485 490 495

Thr Thr Leu Ser Ala Thr Glu Thr Thr Phe Glu Tyr
500 505

INFORMATION FOR SEQ ID NO:2

5 (i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 517

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2

Met Asp Trp Gln Gln Leu Trp Leu Ala Phe Leu Leu Pro Val Thr Val
1 5 10 15

Ser Gly Arg Ala Leu Gly Pro Ala Glu Lys Glu Ala Val Val Asp Tyr
15 20 25 30

Leu Leu Gln Tyr Gly Tyr Leu Gln Lys Pro Leu Glu Gly Ala Asp Asp
35 40 45

Phe Arg Leu Glu Asp Ile Thr Glu Ala Leu Arg Thr Phe Gln Glu Ala
50 55 60

20 Ser Glu Leu Pro Val Ser Gly Gln Met Asp Asp Ala Thr Arg Ala Arg
65 70 75 80

Met Lys Gln Pro Arg Cys Gly Leu Glu Asp Pro Phe Asn Gln Lys Thr
85 90 95

Leu Lys Tyr Leu Leu Gly His Trp Arg Lys Lys His Leu Thr Phe
25 100 105 110

Arg Ile Leu Asn Val Pro Ser Thr Leu Ser Pro Ser Arg Val Arg Ala
115 120 125

Ala Leu His Gln Ala Phe Lys Tyr Trp Ser Asn Val Ala Pro Leu Thr
130 135 140

30 Phe Arg Glu Val Lys Ala Gly Trp Ala Asp Ile Arg Leu Ser Phe His
145 150 155 160

Gly Arg Gln Ser Pro Tyr Cys Ser Asn Ser Phe Asp Gly Pro Gly Lys
165 170 175

Val Leu Ala His Ala Asp Val Pro Glu Leu Gly Ser Val His Phe Asp
35 180 185 190

Asn Asp Glu Phe Trp Thr Glu Gly Thr Tyr Gln Gly Val Asn Leu Arg

	195	200	205
	Ile Ile Ala Ala His Glu Val Gly His Ala Leu Gly Leu Gly His Ser		
	210	215	220
	Arg Tyr Thr Gln Ala Leu Met Ala Pro Val Tyr Ala Gly Tyr Gln Pro		
5	225	230	235
	Tyr Phe Arg Leu His Pro Asp Asp Val Ala Gly Ile Gln Ala Leu Tyr		
	245	250	255
	Gly Lys Arg Arg Pro Glu Pro Glu Asp Glu Glu Glu Val Glu Met		
	260	265	270
10	His Thr Val Ser Thr Val Thr Thr Lys Pro Ser Pro Met Pro Asn Pro		
	275	280	285
	Cys Ser Ser Glu Val Asp Ala Met Met Leu Gly Pro Arg Gly Lys Thr		
	290	295	300
	Tyr Ala Phe Lys Gly Asp Tyr Val Trp Thr Val Thr Asp Ser Gly Pro		
15	305	310	315
	Gly Pro Leu Phe Arg Val Ser Ala Leu Trp Glu Gly Leu Pro Gly Asn		
	325	330	335
	Leu Asp Ala Ala Val Tyr Ser Pro Arg Thr Gln Arg Thr His Phe Phe		
	340	345	350
20	Lys Gly Asn Lys Val Trp Arg Tyr Val Asp Phe Lys Leu Ser Pro Gly		
	355	360	365
	Phe Pro Met Lys Leu Asn Arg Val Glu Pro Asn Leu Asp Ala Ala Leu		
	370	375	380
	Tyr Trp Pro Val Asn Gln Lys Val Phe Leu Phe Lys Gly Ser Gly Tyr		
25	385	390	395
	Trp Gln Trp Asp Glu Leu Thr Arg Thr Asp Leu Ser Arg Tyr Pro Lys		
	405	410	415
	Pro Ile Lys Glu Leu Phe Thr Gly Val Pro Asp Gln Pro Ser Ala Ala		
	420	425	430
30	Met Ser Trp Gln Asp Gly Gln Val Tyr Phe Phe Lys Gly Lys Glu Tyr		
	435	440	445
	Trp Arg Leu Asn Gln Gln Leu Arg Val Ala Lys Gly Tyr Pro Arg Asn		
	450	455	460
	Thr Thr His Trp Met His Cys Ser Pro Arg Thr Pro Asp Thr Asn Ser		
35	465	470	475
	Leu Thr Gly Asp Val Thr Thr Pro Ala Thr Val Glu Ser Val Leu Asp		

485	490	495
Val Pro Ser Ala Thr Asp Ala Ala Ser Leu Ser Ser Ser Ala Asn Val		
500	505	510
Thr Leu Leu Gly Ala		
5	515	

INFORMATION FOR SEQ ID NO:3

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 411

10 (B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3

15	Tyr Leu Leu Leu Gly Arg Trp Arg Lys Lys His Leu Thr Phe Arg Ile	
1	5	10
		15
	Leu Asn Leu Pro Ser Thr Leu Pro Pro His Thr Ala Arg Ala Ala Leu	
	20	25
		30
	Arg Gln Ala Phe Gln Asp Trp Ser Asn Val Ala Pro Leu Thr Phe Gln	
20	35	40
		45
	Glu Val Gln Ala Gly Ala Ala Asp Ile Arg Leu Ser Phe His Gly Arg	
	50	55
		60
	Gln Ser Ser Tyr Cys Ser Asn Thr Phe Asp Gly Pro Gly Arg Val Leu	
	65	70
		75
	Ala His Ala Asp Ile Pro Glu Leu Gly Ser Val His Phe Asp Glu Asp	
25	85	90
		95
	Glu Phe Trp Thr Glu Gly Thr Tyr Arg Gly Val Asn Leu Arg Ile Ile	
	100	105
		110
	Ala Ala His Glu Val Gly His Ala Leu Gly Leu Gly His Ser Arg Tyr	
30	115	120
		125
	Ser Gln Ala Leu Met Ala Pro Val Tyr Glu Gly Tyr Arg Pro His Phe	
	130	135
		140
	Lys Leu His Pro Asp Asp Val Ala Gly Ile Gln Ala Leu Tyr Gly Lys	
	145	150
		155
	160	
35	Lys Ser Pro Val Ile Arg Asp Glu Glu Glu Glu Thr Glu Leu Pro	
	165	170
		175

	Thr Val Pro Pro Val Pro Thr Glu Pro Ser Pro Met Pro Asp Pro Cys		
	180	185	190
	Ser Ser Glu Leu Asp Ala Met Met Leu Gly Pro Arg Gly Lys Thr Tyr		
	195	200	205
5	Ala Phe Lys Gly Asp Tyr Val Trp Thr Val Ser Asp Ser Gly Pro Gly		
	210	215	220
	Pro Leu Phe Arg Val Ser Ala Leu Trp Glu Gly Leu Pro Gly Asn Leu		
	225	230	235
	Asp Ala Ala Val Tyr Ser Pro Arg Thr Gln Trp Ile His Phe Phe Lys		
10	245	250	255
	Gly Asp Lys Val Trp Arg Tyr Ile Asn Phe Lys Met Ser Pro Gly Phe		
	260	265	270
	Pro Lys Lys Leu Asn Arg Val Glu Pro Asn Leu Asp Ala Ala Leu Tyr		
	275	280	285
15	Trp Pro Leu Asn Gln Lys Val Phe Leu Phe Lys Gly Ser Gly Tyr Trp		
	290	295	300
	Gln Trp Asp Glu Leu Ala Arg Thr Asp Phe Ser Ser Tyr Pro Lys Pro		
	305	310	315
	Ile Lys Gly Leu Phe Thr Gly Val Pro Asn Gln Pro Ser Ala Ala Met		
20	325	330	335
	Ser Trp Gln Asp Gly Arg Val Tyr Phe Phe Lys Gly Lys Val Tyr Trp		
	340	345	350
	Arg Leu Asn Gln Gln Leu Arg Val Glu Lys Gly Tyr Pro Arg Asn Ile		
	355	360	365
25	Ser His Asn Trp Met His Cys Arg Pro Arg Thr Ile Asp Thr Thr Pro		
	370	375	380
	Ser Gly Gly Asn Thr Thr Pro Ser Gly Thr Gly Ile Thr Leu Asp Thr		
	385	390	395
	Thr Leu Ser Ala Thr Glu Thr Thr Phe Glu Tyr		
30	405	410	

INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 416

35 (B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4

Gln Lys Thr Leu Lys Tyr Leu Leu Leu Gly Arg Trp Arg Lys Lys His
5 1 5 10 15
Leu Thr Phe Arg Ile Leu Asn Leu Pro Ser Thr Leu Pro Pro His Thr
20 25 30
Ala Arg Ala Ala Leu Arg Gln Ala Phe Gln Asp Trp Ser Asn Val Ala
35 40 45
10 Pro Leu Thr Phe Gln Glu Val Gln Ala Gly Ala Ala Asp Ile Arg Leu
50 55 60
Ser Phe His Gly Arg Gln Ser Ser Tyr Cys Ser Asn Thr Phe Asp Gly
65 70 75 80
Pro Gly Arg Val Leu Ala His Ala Asp Ile Pro Glu Leu Gly Ser Val
15 85 90 95
His Phe Asp Glu Asp Glu Phe Trp Thr Glu Gly Thr Tyr Arg Gly Val
100 105 110
Asn Leu Arg Ile Ile Ala Ala His Glu Val Gly His Ala Leu Gly Leu
115 120 125
20 Gly His Ser Arg Tyr Ser Gln Ala Leu Met Ala Pro Val Tyr Glu Gly
130 135 140
Tyr Arg Pro His Phe Lys Leu His Pro Asp Asp Val Ala Gly Ile Gln
145 150 155 160
Ala Leu Tyr Gly Lys Lys Ser Pro Val Ile Arg Asp Glu Glu Glu
25 165 170 175
Glu Thr Glu Leu Pro Thr Val Pro Pro Val Pro Thr Glu Pro Ser Pro
180 185 190
Met Pro Asp Pro Cys Ser Ser Glu Leu Asp Ala Met Met Leu Gly Pro
195 200 205
30 Arg Gly Lys Thr Tyr Ala Phe Lys Gly Asp Tyr Val Trp Thr Val Ser
210 215 220
Asp Ser Gly Pro Gly Pro Leu Phe Arg Val Ser Ala Leu Trp Glu Gly
225 230 235 240
Leu Pro Gly Asn Leu Asp Ala Ala Val Tyr Ser Pro Arg Thr Gln Trp
35 245 250 255
Ile His Phe Phe Lys Gly Asp Lys Val Trp Arg Tyr Ile Asn Phe Lys

	260	265	270
	Met Ser Pro Gly Phe Pro Lys Lys Leu Asn Arg Val Glu Pro Asn Leu		
	275	280	285
	Asp Ala Ala Leu Tyr Trp Pro Leu Asn Gln Lys Val Phe Leu Phe Lys		
5	290	295	300
	Gly Ser Gly Tyr Trp Gln Trp Asp Glu Leu Ala Arg Thr Asp Phe Ser		
	305	310	315
	Ser Tyr Pro Lys Pro Ile Lys Gly Leu Phe Thr Gly Val Pro Asn Gln		
	325	330	335
10	Pro Ser Ala Ala Met Ser Trp Gln Asp Gly Arg Val Tyr Phe Phe Lys		
	340	345	350
	Gly Lys Val Tyr Trp Arg Leu Asn Gln Gln Leu Arg Val Glu Lys Gly		
	355	360	365
	Tyr Pro Arg Asn Ile Ser His Asn Trp Met His Cys Arg Pro Arg Thr		
15	370	375	380
	Ile Asp Thr Thr Pro Ser Gly Gly Asn Thr Thr Pro Ser Gly Thr Gly		
	385	390	395
	Ile Thr Leu Asp Thr Thr Leu Ser Ala Thr Glu Thr Thr Phe Glu Tyr		
	405	410	415
20			

INFORMATION FOR SEQ ID NO:5

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 419

(B) TYPE: Amino acid

25 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5

	Tyr Leu Leu Leu Gly His Trp Arg Lys Lys His Leu Thr Phe Arg Ile		
30	1	5	10
	Leu Asn Val Pro Ser Thr Leu Ser Pro Ser Arg Val Arg Ala Ala Leu		
	20	25	30
	His Gln Ala Phe Lys Tyr Trp Ser Asn Val Ala Pro Leu Thr Phe Arg		
	35	40	45
35	Glu Val Lys Ala Gly Trp Ala Asp Ile Arg Leu Ser Phe His Gly Arg		
	50	55	60

Gln Ser Pro Tyr Cys Ser Asn Ser Phe Asp Gly Pro Gly Lys Val Leu
 65 70 75 80
 Ala His Ala Asp Val Pro Glu Leu Gly Ser Val His Phe Asp Asn Asp
 85 90 95
 5 Glu Phe Trp Thr Glu Gly Thr Tyr Gln Gly Val Asn Leu Arg Ile Ile
 100 105 110
 Ala Ala His Glu Val Gly His Ala Leu Gly Leu Gly His Ser Arg Tyr
 115 120 125
 Thr Gln Ala Leu Met Ala Pro Val Tyr Ala Gly Tyr Gln Pro Tyr Phe
 10 130 135 140
 Arg Leu His Pro Asp Asp Val Ala Gly Ile Gln Ala Leu Tyr Gly Lys
 145 150 155 160
 Arg Arg Pro Glu Pro Glu Asp Glu Glu Glu Val Glu Met His Thr
 165 170 175
 15 Val Ser Thr Val Thr Thr Lys Pro Ser Pro Met Pro Asn Pro Cys Ser
 180 185 190
 Ser Glu Val Asp Ala Met Met Leu Gly Pro Arg Gly Lys Thr Tyr Ala
 195 200 205
 Phe Lys Gly Asp Tyr Val Trp Thr Val Thr Asp Ser Gly Pro Gly Pro
 20 210 215 220
 Leu Phe Arg Val Ser Ala Leu Trp Glu Gly Leu Pro Gly Asn Leu Asp
 225 230 235 240
 Ala Ala Val Tyr Ser Pro Arg Thr Gln Arg Thr His Phe Phe Lys Gly
 245 250 255
 25 Asn Lys Val Trp Arg Tyr Val Asp Phe Lys Leu Ser Pro Gly Phe Pro
 260 265 270
 Met Lys Leu Asn Arg Val Glu Pro Asn Leu Asp Ala Ala Leu Tyr Trp
 275 280 285
 Pro Val Asn Gln Lys Val Phe Leu Phe Lys Gly Ser Gly Tyr Trp Gln
 30 290 295 300
 Trp Asp Glu Leu Thr Arg Thr Asp Leu Ser Arg Tyr Pro Lys Pro Ile
 305 310 315 320
 Lys Glu Leu Phe Thr Gly Val Pro Asp Gln Pro Ser Ala Ala Met Ser
 325 330 335
 35 Trp Gln Asp Gly Gln Val Tyr Phe Phe Lys Gly Lys Glu Tyr Trp Arg
 340 345 350

Leu Asn Gln Gln Leu Arg Val Ala Lys Gly Tyr Pro Arg Asn Thr Thr
 355 360 365
 His Trp Met His Cys Ser Pro Arg Thr Pro Asp Thr Asn Ser Leu Thr
 370 375 380
 5 Gly Asp Val Thr Thr Pro Ala Thr Val Glu Ser Val Leu Asp Val Pro
 385 390 395 400
 Ser Ala Thr Asp Ala Ala Ser Leu Ser Ser Ser Ala Asn Val Thr Leu
 405 410 415
 Leu Gly Ala
 10
 INFORMATION FOR SEQ ID NO:6
 (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 424
 (B) TYPE: Amino acid
 15 (C) TOPOLOGY: Linear
 (ii) MOLECULE TYPE: Protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6

Gln Lys Thr Leu Lys Tyr Leu Leu Leu Gly His Trp Arg Lys Lys His
 20 1 5 10 15
 Leu Thr Phe Arg Ile Leu Asn Val Pro Ser Thr Leu Ser Pro Ser Arg
 20 25 30
 Val Arg Ala Ala Leu His Gln Ala Phe Lys Tyr Trp Ser Asn Val Ala
 35 40 45
 25 Pro Leu Thr Phe Arg Glu Val Lys Ala Gly Trp Ala Asp Ile Arg Leu
 50 55 60
 Ser Phe His Gly Arg Gln Ser Pro Tyr Cys Ser Asn Ser Phe Asp Gly
 65 70 75 80
 Pro Gly Lys Val Leu Ala His Ala Asp Val Pro Glu Leu Gly Ser Val
 80 85 90 95
 His Phe Asp Asn Asp Glu Phe Trp Thr Glu Gly Thr Tyr Gln Gly Val
 100 105 110
 Asn Leu Arg Ile Ile Ala Ala His Glu Val Gly His Ala Leu Gly Leu
 115 120 125
 35 Gly His Ser Arg Tyr Thr Gln Ala Leu Met Ala Pro Val Tyr Ala Gly
 130 135 140

Tyr Gln Pro Tyr Phe Arg Leu His Pro Asp Asp Val Ala Gly Ile Gln
 145 150 155 160
 Ala Leu Tyr Gly Lys Arg Arg Pro Glu Pro Glu Asp Glu Glu Glu
 165 170 175
 5 Val Glu Met His Thr Val Ser Thr Val Thr Thr Lys Pro Ser Pro Met
 180 185 190
 Pro Asn Pro Cys Ser Ser Glu Val Asp Ala Met Met Leu Gly Pro Arg
 195 200 205
 Gly Lys Thr Tyr Ala Phe Lys Gly Asp Tyr Val Trp Thr Val Thr Asp
 10 210 215 220
 Ser Gly Pro Gly Pro Leu Phe Arg Val Ser Ala Leu Trp Glu Gly Leu
 225 230 235 240
 Pro Gly Asn Leu Asp Ala Ala Val Tyr Ser Pro Arg Thr Gln Arg Thr
 245 250 255
 15 His Phe Phe Lys Gly Asn Lys Val Trp Arg Tyr Val Asp Phe Lys Leu
 260 265 270
 Ser Pro Gly Phe Pro Met Lys Leu Asn Arg Val Glu Pro Asn Leu Asp
 275 280 285
 Ala Ala Leu Tyr Trp Pro Val Asn Gln Lys Val Phe Leu Phe Lys Gly
 20 290 295 300
 Ser Gly Tyr Trp Gln Trp Asp Glu Leu Thr Arg Thr Asp Leu Ser Arg
 305 310 315 320
 Tyr Pro Lys Pro Ile Lys Glu Leu Phe Thr Gly Val Pro Asp Gln Pro
 325 330 335
 25 Ser Ala Ala Met Ser Trp Gln Asp Gly Gln Val Tyr Phe Phe Lys Gly
 340 345 350
 Lys Glu Tyr Trp Arg Leu Asn Gln Gln Leu Arg Val Ala Lys Gly Tyr
 355 360 365
 Pro Arg Asn Thr Thr His Trp Met His Cys Ser Pro Arg Thr Pro Asp
 30 370 375 380
 Thr Asn Ser Leu Thr Gly Asp Val Thr Thr Pro Ala Thr Val Glu Ser
 385 390 395 400
 Val Leu Asp Val Pro Ser Ala Thr Asp Ala Ala Ser Leu Ser Ser Ser
 405 410 415
 35 Ala Asn Val Thr Leu Leu Gly Ala
 420

INFORMATION FOR SEQ ID NO:7

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1524

5 (B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7

10	ATGAACTGCC	AGCAGCTGTG	GCTGGGCTTC	CTACTCCCCA	TGACAGTCTC	AGGCCGGGTC	60
	CTGGGGCTTG	CAGAGGTGGC	CCCCGTGGAC	TACCTGTAC	AATATGGGTA	CCTACAGAAG	120
	CCTCTAGAAG	GATCTAATAA	CTTCAAGCCA	GAAGATATCA	CCGAGGCTCT	GAGAGCTTT	180
	CAGGAAGCAT	CTGAACCTCC	AGTCTCAGGT	CAGCTGGATG	ATGCCACAAAG	GGCCCGCATG	240
15	AGGCAGCCTC	GTTGTGGCCT	AGAGGATCCC	TTCAACCAGA	AGACCCTAA	ATACCTGTTG	300
	CTGGGGCGCT	GGAGAAAGAA	GCACCTGACT	TTCCGCATCT	TGAACCTGCC	CTCCACCCCTT	360
	CCACCCCCACA	CAGCCCCGGC	AGCCCTGCGT	CAAGCCTCC	AGGACTGGAG	CAATGTGGCT	420
	CCCTTGACCT	TCCAAGAGGT	GCAGGCTGGT	GCAGGCTGACA	TCCGCCTCTC	CTTCCATGGC	480
	CGCCAAAGCT	CGTACTGTTC	CAATACTTTT	GATGGGCCTG	GGAGAGTTCT	GGCCCATGCC	540
20	GACATCCCAG	AGCTGGGCAG	TGTGCACCTC	GACGAAGACG	AGTTCTGGAC	TGAGGGGACC	600
	TACCGTGGGG	TGAACCTGCG	CATCATTGCA	GCCCCATGAAG	TGGGCCATGC	TCTGGGGCTT	660
	GGGCACCTCCC	GATATTCCCA	GGCCCTCATG	GCCCCAGTCT	ACGAGGGCTA	CCGGCCCCAC	720
	TTTAAGCTGC	ACCCAGATGA	TGTGGCAGGG	ATCCAGGCTC	TCTATGGCAA	GAAGAGTCCA	780
	GTGATAAGGG	ATGAGGAAGA	AGAAGAGACA	GAGCTGCCA	CTGTGCCCCC	AGTGCCACAA	840
25	GAACCCAGTC	CCATGCCAGA	CCCTTGCAGT	AGTAACTGG	ATGCCATGAT	GCTGGGGCCC	900
	CGTGGGAAGA	CCTATGCTTT	CAAGGGGGAC	TATGTGTGGA	CTGTATGAGA	TTCAGGACCG	960
	GGCCCCCTGT	TCCGAGTGTC	TGCCCTTGG	GAGGGGCTCC	CCGGAAACCT	GGATGCTGCT	1020
	GTCTACTCGC	CTCGAACACA	ATGGATTAC	TTCTTTAAGG	GAGACAAGGT	GTGGCGCTAC	1080
	ATTAATTTCA	AGATGTCTCC	TGGCTTCCCC	AAGAAGCTGA	ATAGGGTAGA	ACCTAACCTG	1140
30	GATGCAGCTC	TCTATTGGCC	TCTCAACCAA	AAGGTGTTCC	TCTTTAAGGG	CTCCGGGTAC	1200
	TGGCAGTGGG	ACGAGCTAGC	CCGAACGTAC	TTCAGCAGCT	ACCCCAAACC	AATCAAGGGT	1260
	TTGTTTACGG	GAGTGCCAAA	CCAGCCCTCG	GCTGCTATGA	GTTGGCAAGA	TGGCCGAGTC	1320
	TACTTCTTC	AGGGCAAAGT	CTACTGGCGC	CTCAACCAAGC	AGCTTCGAGT	AGAGAAAGGC	1380
	TATCCCAAGAA	ATATTTCCCA	CAACTGGATG	CACTGTGTC	CCCGGACTAT	AGACACTACC	1440
35	CCATCAGGTG	GGAATACCAAC	TCCCTCAGGT	ACGGGCATAA	CCTTGGATAC	CACTCTCTCA	1500
	GCCACAGAAA	CCACGTTTG	ATAC				1524

INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1551

(B) TYPE: Nucleic acid

5 (C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8

10	ATGGACTGGC AGCAGCTGTG GCTGGCCTTC TTACTTCCTG TGACAGTCTC AGGCCGGGCT	60
	CTGGGGCCTG CAGAGAAGGA GGCGGTGGTG GATTACCTGT TGCAGTATGG GTATCTACAG	120
	AAACCTCTGG AAGGAGCTGA TGACTTCAGG CTAGAAGATA TCACAGAGGC TCTAAGAACT	180
	TTCCAGGAAG CATCTGAACT GCCTGTTCC GGTCAGATGG ATGATGCCAC AAGGGCCCGT	240
	ATGAAGCAGC CCCGTTGTGG CCTGGAGGAT CCTTTCAACC AGAAGACTCT GAAATACCTG	300
15	CTTCTGGGCC ACTGGAGAAA GAAGCACTTG ACATTCCGCA TCTTGAACGT GCCCTCCACC	360
	CTCTCACCCCT CCAGAGTCCG AGCAGCCCTG CATCAAGCCT TTAAGTATTG GAGCAATGTA	420
	GCCCCCCTGA CCTTCCGGGA GGTAAAGCT GGTTGGGCTG ATATCCGCCT CTCGTTCCAT	480
	GGCCGCCAAA GCCCATACTG CTCCAACAGC TTGATGGGC CTGGGAAGGT CCTGGCCCAT	540
	GCTGACGTCC CAGAGCTTGG CAGTGTACAC TTGATAACG ATGAATTCTG GACCGAGGGC	600
20	ACCTACCAGG GAGTGAACCT ACGCATCATT GCGGCCCATG AGGTGGGCCA CGCCCTGGGA	660
	CTTGGGCATT CCCGATATAC CCAGGCACTC ATGGCGCCTG TTTACGCTGG CTACCAGCCC	720
	TACTTCAGGC TGCATCCGGA TGATGTGGCA GGGATCCAGG CGCTCTATGG CAAGAGGAGG	780
	CCGGAGCCAG AAGATGAGGA GGAAGAGGTG GAGATGCACA CTGTGTCAAC AGTGACCACA	840
	AAACCCAGTC CCATGCCAAA CCCCTGCAGC AGTGAAGTGG ATGCCATGAT GCTAGGGCCT	900
25	CGGGGGAAGA CCTATGCTT CAAGGGTGAC TATGTGTGGA CTGTAACAGA TTCAGGGCCA	960
	GGCCCCCTGT TCCGAGTGTG TGCCCTTTGG GAGGGGCTTC CTGGAAACCT GGATGCTGCT	1020
	GTCTACTCTC CCCGGACACA GCGGACTCAT TTCTTCAAGG GAAACAAGGT GTGGCGGTAT	1080
	GTGGATTTCAGTGTCTCC TGGCTTTCCC ATGAAACTCA ACAGAGTGG ACCAACCTA	1140
	GATGCAGCTC TCTATTGGCC TGTTAACAG AAGGTGTTCC TTTTTAAGGG CTCAGGATAC	1200
30	TGGCAATGGG ATGAACGTGAC CAGAACTGAC CTCAGTCGCT ACCCCAAACC AATCAAGGAA	1260
	CTTTCACTG GAGTGCCAGA CCAACCCCTCA GCAGCTATGA GCTGGCAAGA TGCCCAAGTC	1320
	TACTTCTTCAGAAGGAA GAAACTGGCGC CTTAACCCAGC AACCTCGAGT GGAAAGGGC	1380
	TATCCCCAGAA ATACGACACA CTGGATGCAC TGAGTCCTC GGACTCCAGA CACTAACTCA	1440
	TTAAGTGGGG ATGTGACCAAC TCCTGCAACC GTGGAATGAG TCTTGGATGT TCCCTCTGCC	1500
35	ACAGACGCTG CCTCCCTCTC ATCCTCAGCT AATGTCACCT TGCTAGGGC C	1551

INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1233

(B) TYPE: Nucleic acid

5 (C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

10	TACCTGTTGC TGGGCCGCTG GAGAAAGAAG CACCTGACTT TCCGCATCTT GAACCTGCC	60
	TCCACCCCTTC CACCCCACAC AGCCCGGGCA GCCCTGCGTC AAGCCTTCCA GGACTGGAGC	120
	AATGTGGCTC CCTTGACCTT CCAAGAGGTG CAGGCTGGTG CGGCTGACAT CCGCCTCTCC	180
	TTCCATGGCC GCCAAAGCTC GTACTGTCC AATACTTTTG ATGGGCCTGG GAGAGTTCTG	240
	GCCCCATGCCG ACATCCCAGA GCTGGGCAGT GTGCACCTTCG ACGAAGACGA GTTCTGGACT	300
15	GAGGGGACCT ACCGTGGGGT GAACCTGCGC ATCATTGCAG CCCATGAAGT GGGCCATGCT	360
	CTGGGGCTTG GGCACCTCCCG ATATTCCAG GCCCTCATGG CCCCAGTCTA CGAGGGCTAC	420
	CGGGCCCCACT TTAAGCTGCA CCCAGATGAT GTGGCAGGGA TCCAGGCTCT CTATGGCAAG	480
	AAGAGTCCAG TGATAAGGGA TGAGGAAGAA GAAGAGACAG AGCTGCCAC TGTCCCCCA	540
	GTGCCACACAG AACCCAGTCC CATGCCAGAC CCTTGCAGTA GTGAACTGGA TGCCATGATG	600
20	CTGGGGCCCC GTGGGAAGAC CTATGTTTC AAGGGGGACT ATGTGTGGAC TGTATCAGAT	660
	TCAGGACCGG GCCCCTTGTG CCGAGTGTCT GCCCTTTGGG AGGGGCTCCC CGGAAACCTG	720
	GATGCTGCTG TCTACTCGCC TCGAACACAA TGGATTCACT TCTTTAAGGG AGACAAGGTG	780
	TGGCGCTACA TTAATTCAA GATGTCTCTT GGCTTCCCCA AGAAGCTGAA TAGGGTAGAA	840
	CCTAACCTGG ATGCAGCTCT CTATTGGCT CTCAACCAAA AGGTGTTCTT CTTTAAGGGC	900
25	TCCGGGTACT GGCACTGGGA CGAGCTAGCC CGAACTGACT TCAGCAGCTA CCCCCAAACCA	960
	ATCAAGGGTT TGTTTACGGG AGTGCCAAC CAGCCCTCGG CTGCTATGAG TTGGCAAGAT	1020
	GGCCGAGTCT ACTTCTTCAA GGGCAAAGTC TACTGGCGCC TCAACCAGCA GCTTCGAGTA	1080
	GAGAAAGGCT ATCCCAGAAA TATTCCCAC AACTGGATGC ACTGTGTCCTT CCGGACTATA	1140
	GACACTACCC CATCAGGTGG GAATACCACT CCCTCAGGTA CGGGCATAAC CTTGGATACC	1200
30	ACTCTCTCAG CCACAGAAAC CACGTTGAA TAC	1233

INFORMATION FOR SEQ ID NO:10

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1248

35 (B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear
 (ii) MOLECULE TYPE: cDNA
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10

5	CAGAAGACCC TTAAATACCT GTTGCTGGGC CGCTGGAGAA AGAACGACCT GACTTCCGC	60
	ATCTGAACC TGCCCTCCAC CCTTCCACCC CACACAGCCC GGGCAGCCCT GCGTCAGCC	120
	TTCCAGGACT GGAGCAATGT GGCTCCCTTG ACCTTCCAAG AGGTGCAGGC TGGTGCAGCT	180
	GACATCCGCC TCTCCTTCCA TGGCCGCCAA AGCTCGTACT GTTCCAATAC TTTTGATGGG	240
	CCTGGGAGAG TTCTGGCCA TGCCGACATC CCAGAGCTGG GCAGTGTGCA CTTCGACGAA	300
10	GACGAGTTCT GGACTGAGGG GACCTACCGT GGGGTGAACC TGCGCATCAT TGCAGCCCAT	360
	GAAGTGGGCC ATGCTCTGGG GCTTGGGCAC TCCCGATATT CCCAGGCCCT CATGGCCCCA	420
	GTCTACGAGG GCTACCGGCC CCACCTTAAG CTGCACCCAG ATGATGTGGC AGGGATCCAG	480
	GCTCTCTATG GCAAGAAGAG TCCAGTGATA AGGGATGAGG AAGAAGAAGA GACAGAGCTG	540
	CCCACTGTGC CCCCAGTGCC CACAGAACCC AGTCCCATGC CAGACCCCTG CAGTAGTGAA	600
15	CTGGATGCCA TGATGCTGGG GCCCCGTGGG AAGACCTATG CTTTCAAGGG GGACTATGTG	660
	TGGACTGTAT CAGATTCAAGG ACCGGGCCCC TTGTTCCGAG TGTCTGCCCT TTGGGAGGGG	720
	CTCCCCGGAA ACCTGGATGC TGCTGTCTAC TCGCCTCGAA CACAATGGAT TCACTTCTTT	780
	AAGGGAGACA AGGTGTGGCG CTACATTAAT TTCAAGATGT CTCCTGGCTT CCCCAAGAAG	840
	CTGAATAGGG TAGAACCTAA CCTGGATGCA GCTCTCTATT GGCTCTCAA CCAAAAGGTG	900
20	TTCCCTTTA AGGGCTCCGG GTACTGGCAG TGGGACGAGC TAGCCGAAC TGACTTCAGC	960
	AGCTACCCCA AACCAATCAA GGGTTTGTTC ACGGGAGTGC CAAACCCAGCC CTCGGCTGCT	1020
	ATGAGTTGGC AAGATGGCCG AGTCTACTTC TTCAAGGGCA AAGTCTACTG GCGCCTAAC	1080
	CAGCAGCTTC GAGTAGAGAA AGGCTATCCC AGAAATATTG CCCACAACTG GATGCACTGT	1140
	CGTCCCCGGA CTATAGACAC TACCCCATCA GGTGGGAATA CCACTCCCTC AGGTACGGGC	1200
25	ATAACCTTGG ATACCACTCT CTCAGGCCACA GAAACCACGT TTGAATAC	1248

INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1257

30 (B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11

	TCCACCCCT CACCCCTCCAG AGTCCGAGCA GCCCTGCATC AAGCCTTAA GTATTGGAGC	120
	AATGTAGCCC CCCTGACCTT CCGGGAGGTG AAAGCTGGTT GGGCTGATAT CCGCCTCTCG	180
	TTCCATGGCC GCCAAAGCCC ATACTGCTCC AACAGCTTG ATGGGCCTGG GAAGGTCCGT	240
	GCCCATGCTG ACGTCCCAGA GCTTGGCAGT GTACACTTCG ATAACGATGA ATTCTGGACC	300
5	GAGGGCACCT ACCAGGGAGT GAACCTACGC ATCATTGCGG CCCATGAGGT GGGCCACGCC	360
	CTGGGACTTG GGCATTCCCCG ATATACCCAG GCACTCATGG CGCCTGTTA CGCTGGCTAC	420
	CAGCCCTACT TCAGGCTGCA TCCGGATGAT GTGGCAGGGA TCCAGGGCT CTATGGCAAG	480
	AGGAGGCCGG AGCCAGAAGA TGAGGAGGAA GAGGTGGAGA TGCACACTGT GTCAACAGTG	540
	ACCACAAAAC CCAGTCCCCT GCCAAACCCC TGCAGCAGTG AAGTGGATGC CATGATGCTA	600
10	GGGCCTCGGG GGAAGACCTA TGCTTCAAG GGTGACTATG TGTGGACTGT AACAGATTCA	660
	GGGCCAGGGC CCTTGTTCGG AGTGTCTGCC CTTTGGGAGG GGCTTCCTGG AAACCTGGAT	720
	GCTGCTGTCT ACTCTCCCCG GACACAGCGG ACTCATTCT TCAAGGGAAA CAAGGTGTGG	780
	CGGTATGTGG ATTTCAAGTT GTCTCCTGGC TTTCCATGA AACTCAACAG AGTGGAACCC	840
	AACCTAGATG CAGCTCTCTA TTGGCCTGTT AATCAGAAGG TGTTCTTT TAAGGGCTCA	900
15	GGATACTGGC AATGGGATGA ACTGACCAGA ACTGACCTCA GTCGCTACCC CAAACCAATC	960
	AAGGAACCTT TCACTGGAGT GCCAGACCAA CCCTCAGCG CTTGAGCTG GCAAGATGGC	1020
	CAAGTCTACT TCTTCAAGGG CAAAGAGTAC TGGCGCCTTA ACCAGCAACT TCGAGTGGCA	1080
	AAGGGCTATC CCAGAAATAC GACACACTGG ATGCACTGTA GTCCTCGGAC TCCAGACACT	1140
	AACTCATTAA CTGGGGATGT GACCACCTCCT GCAACCGTGG AATCAGTCTT GGATGTTCCC	1200
20	TCTGCCACAG ACGCTGCCTC CCTCTCATCC TCAGCTAATG TCACCTTGCT AGGGGCC	1257

INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1272

25 (B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

30	CAGAAGACTC TGAAATACCT GCTTCTGGGC CACTGGAGAA AGAAGCACCT GACATTCCGC	60
	ATCTTGAACG TGCCCTCCAC CCTCTCACCC TCCAGAGTCC GAGCAGCCCT GCATCAAGCC	120
	TTTAAGTATT GGAGCAATGT AGCCCCCTG ACCTTCCGGG AGGTGAAAGC TGGTTGGGCT	180
	GATATCCGCC TCTCGTTCCA TGGCCGCCAA AGCCCATACT GCTCCAACAG CTTTGATGGG	240
35	CCTGGGAAGG TCCTGGCCCA TGCTGACGTC CCAGAGCTTG GCAGTGTACA CTTCGATAAC	300
	GATGAATTCT GGACCGAGGG CACCTACCAAG GGAGTGAACC TACGGCATCAT TGCGGCCAT	360

	GAGGTGGGCC ACGCCCTGGG ACTTGGGCAT TCCCGATATA CCCAGGCCT CATGGCGCT	420
	GTTTACGCTG GCTACCAGCC CTACTTCAGG CTGCATCCGG ATGATGTGGC AGGGATCCAG	480
	GCGCTCTATG GCAAGAGGAG GCGGAGCCA GAAGATGAGG AGGAAGAGGT GGAGATGCAC	540
	ACTGTGTCAA CAGTGACCA AAAACCCAGT CCCATGCCAA ACCCCTGCAG CAGTGAAGTG	600
5	GATGCCATGA TGCTAGGGCC TCGGGGAAG ACCTATGCTT TCAAGGGTGA CTATGTGTGG	660
	ACTGTAACAG ATTCAGGGCC AGGGCCCTTG TTCCGAGTGT CTGCCCTTG GGAGGGGCTT	720
	CCTGGAAACC TGGATGCTGC TGTCTACTCT CCCCGGACAC AGCGGACTCA TTTCTCAAG	780
	GGAAACAAGG TGTGGCGGTA TGTGGATTTC AAGTTGTCTC CTGGCTTCC CATGAAACTC	840
	AACAGAGTGG AACCCAAACCT AGATGCAGCT CTCTATTGGC CTGTTAATCA GAAGGTGTT	900
10	CTTTTAAGG GCTCAGGATA CTGGCAATGG GATGAACTGA CCAGAACTGA CCTCAGTCGC	960
	TACCCCAAAC CAATCAAGGA ACTTTCACT GGAGTGCCAG ACCAACCCCTC AGCAGCTATG	1020
	AGCTGGCAAG ATGGCAAGT CTACTTCTTC AAGGGCAAAG AGTACTGGCG CCTTAACCAG	1080
	CAACCTCGAG TGGCAAAGGG CTATCCCAGA AATACGACAC ACTGGATGCA CTGTAGTCCT	1140
	CGGACTCCAG ACACTAACTC ATTAACTGGG GATGTGACCA CTCCTGCAAC CGTGGAAATCA	1200
15	GTCTGGATG TTCCCTCTGC CACAGACGCT GCCTCCCTCT CATCCTCAGC TAATGTCACC	1260
	TTGCTAGGGG CC	1272

INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS

20	(A) LENGTH: 20
	(B) TYPE: Nucleic acid
	(C) STRANDEDNESS: Single
	(D) TOPOLOGY: Linear
	(ii) MOLECULE TYPE: Synthetic DNA
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13

GCTGACATCC GCCTCTCCTT 20

INFORMATION FOR SEQ ID NO:14

30	(i) SEQUENCE CHARACTERISTICS
	(A) LENGTH: 20
	(B) TYPE: Nucleic acid
	(C) STRANDEDNESS: Single
	(D) TOPOLOGY: Linear
35	(ii) MOLECULE TYPE: Synthetic DNA
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14

GGGCCCCGGTC CTGAAATCTG 20

INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS

5 (A) LENGTH: 29
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(ii) MOLECULE TYPE: Synthetic DNA
10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15

CCCGCATGCT ACCTGTTGCT GGGCCGCTG

INFORMATION FOR SEQ ID NO:16

15 (i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 33
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
20 (ii) MOLECULE TYPE: Synthetic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16

AAGCTGCAGA TCTACGGTCT TGCGCCTGCT ACA

25 INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 19
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
30 (D) TOPOLOGY: Linear
(ii) MOLECULE TYPE: Synthetic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17

GGCAGGGATC CAGGCTCTC 19

35

INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 18
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- 5 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18

TGCATCCAGG TTAGGGTTC 18

10

INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 20
- (B) TYPE: Nucleic acid
- 15 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

- (ii) MOLECULE TYPE: Synthetic DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19

20 GCCGGAGCCA GAAGATGAGG 20

Claims

1. A protein comprising an amino acid sequence represented by SEQ ID NO:1 or a substantially equivalent thereto, or a salt thereof.
2. The protein according to claim 1, which comprises an amino acid sequence represented by SEQ ID NO:2.
3. The protein according to claim 1, which is a metalloprotease.
4. A partial peptide of the protein according to claim 1, or a salt thereof, which shows the activity of the protein according to claim 1.
5. An isolated DNA which contains a DNA comprising a nucleotide sequence coding for a protein according to claim 1.
6. The DNA according to claim 5, which comprises a nucleotide sequence represented by SEQ ID NO:7.
7. The DNA according to claim 5, which comprises a nucleotide sequence represented by SEQ ID NO:8.
8. A recombinant vector comprising the DNA according to claim 5.
9. A transformant carrying the recombinant vector according to claim 8.
10. A process for producing a protein or a salt thereof according to claim 1, which comprises culturing a transformant according to claim 9 under conditions suitable to express said protein.
11. A pharmaceutical composition which comprises the protein according to claim 1 or the partial peptide according to claim 4.
12. The pharmaceutical composition according to claim 11, which is a therapeutic or prophylactic composition for diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk.
13. A pharmaceutical composition which comprises the

DNA according to claim 5.

14. The pharmaceutical composition according to claim 13 which is a therapeutic or prophylactic composition for diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk.

15. An antibody against the protein according to claim 1 or the partial peptide according to claim 4.

16. A method for screening for a compound which activates or inhibits a proteolytic activity of the protein according to claim 1 or the partial peptide according to claim 4, which comprises measuring and comparing a proteolytic activity of the protein according to claim 1 or the partial peptide according to claim 4, in cases of (i) a substrate is contacted with the protein according to claim 1 or the partial peptide according to claim 4 and (ii) a substrate and a test compound are contacted with the protein according to claim 1 or the partial peptide according to claim 4.

17. A kit for screening for a compound which activates or inhibits a proteolytic activity of the protein according to claim 1 or the partial peptide according to claim 4, which comprises the protein according to claim 1 or the partial peptide according to claim 4.

18. A compound which activates a proteolytic activity of the protein according to claim 1 or the partial peptide according to claim 4, which is identified by the screening method according to claim 16 or the kit according to claim 17.

19. A pharmaceutical composition which comprises the compound which inhibits a proteolytic activity of the protein according to claim 1 or the partial peptide according to claim 4, which is identified by the screening method according to claim 16 or the kit according to claim 17.

20. A method for treating or preventing diabetic

nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk in a mammal, which comprises administering to said mammal an effective amount of the protein according to claim 1 or the partial peptide according to claim 4.

21. Use of the protein according to claim 1 or the partial peptide according to claim 4 for production of a therapeutic or prophylactic composition for diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk.

22. A method for treating or preventing diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk in a mammal, which comprises administering an effective amount of the DNA according to claim 5 to the mammal.

23. Use of the DNA according to claim 5 for production of a therapeutic or prophylactic composition for diabetic nephropathy, glomerulonephritis, pulmonary fibrosis, hepatolienal fibrosis, hepatocirrhosis, osteopetrosis or herniated disk.

Fig. 1

Met Asn Cys Gln Gln Leu Trp Leu Gly Phe Leu Leu Pro Met Thr Val Ser Gly Arg Val	20
Leu Gly Leu Ala Glu Val Ala Pro Val Asp Tyr Leu Ser Gln Tyr Gly Tyr Leu Gln Lys	40
Pro Leu Glu Gly Ser Asn Asn Phe Lys Pro Glu Asp Ile Thr Glu Ala Leu Arg Ala Phe	60
Gln Glu Ala Ser Glu Leu Pro Val Ser Gly Gln Leu Asp Asp Ala Thr Arg Ala Arg Met	80
Arg Gln Pro Arg Cys Gly Leu Glu Asp Pro Phe Asn Gln Lys Thr Leu Lys Tyr Leu Leu	100
Leu Gly Arg Trp Arg Lys Lys His Leu Thr Phe Arg Ile Leu Asn Leu Pro Ser Thr Leu	120
Pro Pro His Thr Ala Arg Ala Ala Leu Arg Gln Ala Phe Gln Asp Trp Ser Asn Val Ala	140
Pro Leu Thr Phe Gln Glu Val Gln Ala Gly Ala Ala Asp Ile Arg Leu Ser Phe His Gly	160
Arg Gln Ser Ser Tyr Cys Ser Asn Thr Phe Asp Gly Pro Gly Arg Val Leu Ala His Ala	180
Asp Ile Pro Glu Leu Gly Ser Val His Phe Asp Glu Asp Glu Phe Trp Thr Glu Gly Thr	200
Tyr Arg Gly Val Asn Leu Arg Ile Ile Ala Ala His Glu Val Gly His Ala Leu Gly Leu	220
Gly His Ser Arg Tyr Ser Gln Ala Leu Met Ala Pro Val Tyr Glu Gly Tyr Arg Pro His	240
Phe Lys Leu His Pro Asp Asp Val Ala Gly Ile Gln Ala Leu Tyr Gly Lys Ser Pro	260
Val Ile Arg Asp Glu Glu Glu Glu Thr Glu Leu Pro Thr Val Pro Pro Val Pro Thr	280
Glu Pro Ser Pro Met Pro Asp Pro Cys Ser Ser Glu Leu Asp Ala Met Met Leu Gly Pro	300
Arg Gly Lys Thr Tyr Ala Phe Lys Gly Asp Tyr Val Trp Thr Val Ser Asp Ser Gly Pro	320
Gly Pro Leu Phe Arg Val Ser Ala Leu Trp Glu Gly Leu Pro Gly Asn Leu Asp Ala Ala	340
Val Tyr Ser Pro Arg Thr Gln Trp Ile His Phe Phe Lys Gly Asp Lys Val Trp Arg Tyr	360
Ile Asn Phe Lys Met Ser Pro Gly Phe Pro Lys Lys Leu Asn Arg Val Glu Pro Asn Leu	380
Asp Ala Ala Leu Tyr Trp Pro Leu Asn Gln Lys Val Phe Leu Phe Lys Gly Ser Gly Tyr	400
Trp Gln Trp Asp Glu Leu Ala Arg Thr Asp Phe Ser Ser Tyr Pro Lys Pro Ile Lys Gly	420
Leu Phe Thr Gly Val Pro Asn Gln Pro Ser Ala Ala Met Ser Trp Gln Asp Gly Arg Val	440
Tyr Phe Phe Lys Gly Lys Val Tyr Trp Arg Leu Asn Gln Gln Leu Arg Val Glu Lys Gly	460
Tyr Pro Arg Asn Ile Ser His Asn Trp Met His Cys Arg Pro Arg Thr Ile Asp Thr Thr	480
Pro Ser Gly Gly Asn Thr Thr Pro Ser Gly Thr Gly Ile Thr Leu Asp Thr Thr Leu Ser	500
Ala Thr Glu Thr Thr Phe Glu Tyr	508

Fig. 2

Tyr Leu Leu Leu Gly Arg Trp Arg Lys Lys His Leu Thr Phe Arg Ile Leu Asn Leu Pro	20
Ser Thr Leu Pro Pro His Thr Ala Arg Ala Ala Leu Arg Gln Ala Phe Gln Asp Trp Ser	40
Asn Val Ala Pro Leu Thr Phe Gln Glu Val Gln Ala Gly Ala Ala Asp Ile Arg Leu Ser	60
Phe His Gly Arg Gln Ser Ser Tyr Cys Ser Asn Thr Phe Asp Gly Pro Gly Arg Val Leu	80
Ala His Ala Asp Ile Pro Glu Leu Gly Ser Val His Phe Asp Glu Asp Glu Phe Trp Thr	100
Glu Gly Thr Tyr Arg Gly Val Asn Leu Arg Ile Ile Ala Ala His Glu Val Gly His Ala	120
Leu Gly Leu Gly His Ser Arg Tyr Ser Gln Ala Leu Met Ala Pro Val Tyr Glu Gly Tyr	140
Arg Pro His Phe Lys Leu His Pro Asp Asp Val Ala Gly Ile Gln Ala Leu Tyr Gly Lys	160
Lys Ser Pro Val Ile Arg Asp Glu Glu Glu Glu Thr Glu Leu Pro Thr Val Pro Pro	180
Val Pro Thr Glu Pro Ser Pro Met Pro Asp Pro Cys Ser Ser Glu Leu Asp Ala Met Met	200
Leu Gly Pro Arg Gly Lys Thr Tyr Ala Phe Lys Gly Asp Tyr Val Trp Thr Val Ser Asp	220
Ser Gly Pro Gly Pro Leu Phe Arg Val Ser Ala Leu Trp Glu Gly Leu Pro Gly Asn Leu	240
Asp Ala Ala Val Tyr Ser Pro Arg Thr Gln Trp Ile His Phe Phe Lys Gly Asp Lys Val	260
Trp Arg Tyr Ile Asn Phe Lys Met Ser Pro Gly Phe Pro Lys Lys Leu Asn Arg Val Glu	280
Pro Asn Leu Asp Ala Ala Leu Tyr Trp Pro Leu Asn Gln Lys Val Phe Leu Phe Lys Gly	300
Ser Gly Tyr Trp Gln Trp Asp Glu Leu Ala Arg Thr Asp Phe Ser Ser Tyr Pro Lys Pro	320
Ile Lys Gly Leu Phe Thr Gly Val Pro Asn Gln Pro Ser Ala Ala Met Ser Trp Gln Asp	340
Gly Arg Val Tyr Phe Phe Lys Gly Lys Val Tyr Trp Arg Leu Asn Gln Gln Leu Arg Val	360
Glu Lys Gly Tyr Pro Arg Asn Ile Ser His Asn Trp Met His Cys Arg Pro Arg Thr Ile	380
Asp Thr Thr Pro Ser Gly Gly Asn Thr Thr Pro Ser Gly Thr Gly Ile Thr Leu Asp Thr	400
Thr Leu Ser Ala Thr Glu Thr Thr Phe Glu Tyr	411

Fig. 3

Gln Lys Thr Leu Lys Tyr Leu Leu Leu Gly Arg Trp Arg Lys Lys His Leu Thr Phe Arg	20
Ile Leu Asn Leu Pro Ser Thr Leu Pro Pro His Thr Ala Arg Ala Ala Leu Arg Gln Ala	40
Phe Gln Asp Trp Ser Asn Val Ala Pro Leu Thr Phe Gln Glu Val Gln Ala Gly Ala Ala	60
Asp Ile Arg Leu Ser Phe His Gly Arg Gln Ser Ser Tyr Cys Ser Asn Thr Phe Asp Gly	80
Pro Gly Arg Val Leu Ala His Ala Asp Ile Pro Glu Leu Gly Ser Val His Phe Asp Glu	100
Asp Glu Phe Trp Thr Glu Gly Thr Tyr Arg Gly Val Asn Leu Arg Ile Ile Ala Ala His	120
Glu Val Gly His Ala Leu Gly Leu Gly His Ser Arg Tyr Ser Gln Ala Leu Met Ala Pro	140
Val Tyr Glu Gly Tyr Arg Pro His Phe Lys Leu His Pro Asp Asp Val Ala Gly Ile Gln	160
Ala Leu Tyr Gly Lys Lys Ser Pro Val Ile Arg Asp Glu Glu Glu Glu Thr Glu Leu	180
Pro Thr Val Pro Pro Val Pro Thr Glu Pro Ser Pro Met Pro Asp Pro Cys Ser Ser Glu	200
Leu Asp Ala Met Met Leu Gly Pro Arg Gly Lys Thr Tyr Ala Phe Lys Gly Asp Tyr Val	220
Trp Thr Val Ser Asp Ser Gly Pro Gly Pro Leu Phe Arg Val Ser Ala Leu Trp Glu Gly	240
Leu Pro Gly Asn Leu Asp Ala Ala Val Tyr Ser Pro Arg Thr Gln Trp Ile His Phe Phe	260
Lys Gly Asp Lys Val Trp Arg Tyr Ile Asn Phe Lys Met Ser Pro Gly Phe Pro Lys Lys	280
Leu Asn Arg Val Glu Pro Asn Leu Asp Ala Ala Leu Tyr Trp Pro Leu Asn Gln Lys Val	300
Phe Leu Phe Lys Gly Ser Gly Tyr Trp Gln Trp Asp Glu Leu Ala Arg Thr Asp Phe Ser	320
Ser Tyr Pro Lys Pro Ile Lys Gly Leu Phe Thr Gly Val Pro Asn Gln Pro Ser Ala Ala	340
Met Ser Trp Gln Asp Gly Arg Val Tyr Phe Phe Lys Gly Lys Val Tyr Trp Arg Leu Asn	360
Gln Gln Leu Arg Val Glu Lys Gly Tyr Pro Arg Asn Ile Ser His Asn Trp Met His Cys	380
Arg Pro Arg Thr Ile Asp Thr Pro Ser Gly Gly Asn Thr Thr Pro Ser Gly Thr Gly	400
Ile Thr Leu Asp Thr Thr Leu Ser Ala Thr Glu Thr Thr Phe Glu Tyr	416

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Fig. 4 (Cont.)

10	20	30	40	50
AAGAGCCCTCTGCCTAGCACTGCTCCCCAAGGCTCCAGAAATCTCAG				
60	70	80	90	100
GTCAGAGGCACGGACAGCCTCTGGAGCTCTCGTCTGGTGGGACCATGAAC				
			M	N
110	120	130	140	150
TGCCAGCAGCTGGCTGGCTTCTACTCCCCATGACAGTCTCAGGCCG				
C	Q	Q	L	W
L	W	L	G	F
G	F	L	L	P
M	M	P	M	T
T	V	V	S	S
V	S	G	R	R
160	170	180	190	200
GGTCCTGGGCTTGCAGAGGTGGGCCCGTGGACTACCTGTACAATATG				
V	L	G	L	A
L	A	E	V	A
G	V	A	P	V
S	P	D	Y	L
N	V	Y	L	S
N	Q	Y	S	Q
F	Y	G	Q	Y
210	220	230	240	250
GGTACCTACAGAACGCCTCTAGAAGGATCTAATAACTCAAGGCCAGAACAT				
Y	L	Q	K	P
L	Q	K	P	L
E	G	S	N	N
G	S	N	N	F
S	N	F	K	K
N	F	K	P	E
F	K	P	E	D
260	270	280	290	300
ATCACCGAGGCTCTGAGAGCTTTAGGAAGCATCTGAACCTCCAGTC				
I	T	E	A	L
T	E	A	L	R
E	A	L	R	A
A	F	R	A	F
F	R	A	F	Q
Q	E	A	S	E
E	A	S	E	L
A	S	E	L	P
S	E	L	P	V
310	320	330	340	350
AGGTAGCTGGATGATGCCACAAGGGCCCGCATGAAGGCAGCCTCGTGTG				
G	Q	L	D	D
Q	L	D	D	A
L	D	D	A	T
D	A	T	R	A
D	A	T	R	M
A	T	R	M	R
T	M	R	R	Q
M	R	R	P	P
R	R	P	R	C
R	R	P	R	G
360	370	380	390	400
GCCTAGAGGATCCCTTCAACCAGAAGACCTTAAATACCTGTGCTGGC				
L	E	D	P	F
E	D	P	F	N
D	P	F	N	Q
P	F	N	Q	K
F	N	Q	K	T
N	Q	K	T	L
Q	K	T	L	K
K	T	L	K	Y
T	L	K	Y	L
L	K	Y	L	L
K	Y	L	L	G
410	420	430	440	450
CGCTGGAGAAAGAACCTGACTTCCGCATCTGAACCTGCCCTCCAC				
R	W	R	K	K
W	R	K	K	H
R	K	K	H	L
K	K	H	L	T
K	H	L	T	F
H	L	T	F	R
L	T	F	R	I
T	F	R	I	L
F	R	I	L	N
R	I	L	N	L
I	L	N	L	P
L	N	L	P	S
N	L	P	S	T

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Fig. 4 (Cont.)

460	470	480	490	500
CCTTCCACCCCCACACAGCCCCGGGCAGCCCTGCGTCAAGCCTTCCAGGACT				
L	P	P	H	T
A	R	A	A	L
R	Q	A	F	Q
A	F	Q	D	W
510	520	530	540	550
GGAGGAATGIGGCTCCCTTGACCTTCCAAGAGGTGCAGGCTGGTGGGCT				
S	N	V	A	P
L	T	F	Q	E
T	F	Q	E	V
Q	A	V	Q	A
560	570	580	590	600
GACATCCGCTCTCCATGGCCGCCAAAGCTCGTACTGTCATCCAATAC				
D	I	R	L	S
S	F	H	G	R
F	Q	R	Q	S
S	S	Y	S	Y
Y	C	S	N	T
610	620	630	640	650
TTTGATGGGCTGGGAGAGTTCTGGCCATGCCGACATCCCAGAGCTGG				
F	D	G	P	G
R	V	L	A	H
A	H	A	D	I
D	I	P	E	L
I	P	E	L	G
660	670	680	690	700
GCAGTGTGCACTTCGACGAAGACGAGTTCTGGACTGAGGGACCTACCGT				
S	V	H	F	D
E	D	E	F	W
E	F	W	T	E
G	T	E	G	T
T	Y	R	Y	R
710	720	730	740	750
GGGGTGAACCTGCGCATCATTGCAGCCCATGAAGTGGCCATGCTCTGGG				
G	V	N	L	R
I	I	A	A	H
A	A	H	E	V
H	E	V	G	H
A	L	G	A	L
760	770	780	790	800
GCTGGGCACTCCCGATATTCCCAAGGCCCTCATGGCCCCAGTCTACGAGG				
L	G	H	S	R
S	R	Y	S	Q
Y	S	Q	A	L
A	L	M	A	P
M	A	P	V	Y
P	V	Y	E	G
810	820	830	840	850
GCTACCGGGCCCCACTTTAACGCTGCACCCAGATGATGTGGCAGGGATCCAG				
Y	R	P	H	F
R	P	H	F	K
P	H	F	K	L
H	F	K	L	H
F	K	L	H	P
K	L	H	P	D
L	H	P	D	D
H	P	D	D	V
P	D	V	A	G
D	V	A	G	I
V	A	G	I	Q

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Fig. 4 (Cont.)

860	870	880	890	900
GCTCTCTATGGCAAGAAGAGTCCAGTGATAAGGATGAGGAAGAAGAAGA				
A	L	Y	G	K
K	K	S	P	V
I	R	D	E	E
E	E	E	E	E
910	920	930	940	950
GACAGAGCTGCCACTGTGCCCGAGTGCCCCACAGAACCCAGTCCCATGC				
T	E	L	P	T
V	P	P	V	P
P	T	E	P	S
S	P	M	M	P
960	970	980	990	1000
CAGACCCCTTGCAGTAGTGAACGGATGCCATGATGCTGGGGCCCCGG				
D	P	C	S	S
S	E	L	D	A
M	M	M	L	G
L	G	P	R	R
1010	1020	1030	1040	1050
AAGACCTATGCTTCAAGGGGGACTATGTGIGGACTGTATCAGATTGAGG				
K	T	Y	A	F
K	G	D	Y	V
W	T	V	S	D
T	S	S	G	G
1060	1070	1080	1090	1100
ACCGGGGCCCCCTTGTCCGAGTGTCTGCCCTTGGAGGGCTCCCCGGAA				
P	G	P	L	F
R	V	S	A	L
W	E	G	L	P
E	G	N	G	N
1110	1120	1130	1140	1150
ACCTGGATGCTGCTGTACTCGCCTCGAACACAATGGATTCACTTCTTT				
L	D	A	A	V
A	V	Y	S	P
Y	S	P	R	T
S	P	R	T	Q
P	R	T	Q	W
R	T	Q	W	I
T	Q	W	I	H
Q	W	I	H	F
W	I	H	F	F
1160	1170	1180	1190	1200
AAGGGAGACAAGGTGTGGCGCTACATTAATTCAAGATGTCTCTGGCTT				
K	G	D	K	V
D	K	V	W	R
K	V	W	R	Y
V	W	R	Y	I
W	R	Y	I	N
R	Y	I	N	F
Y	I	N	F	K
I	N	F	K	M
N	F	K	M	S
F	K	M	S	P
K	M	S	P	G
M	S	P	G	F
1210	1220	1230	1240	1250
CCCCAAGAAGCTGAATAGGGTAGAACCTAACCTGGATGCAGCTCTATT				
P	K	K	L	N
N	R	V	E	P
R	V	E	P	N
V	E	P	N	L
E	P	N	L	D
P	N	L	D	A
N	L	D	A	A
L	D	A	A	L
D	A	A	L	Y
A	A	L	Y	W

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Fig. 4 (Cont.)

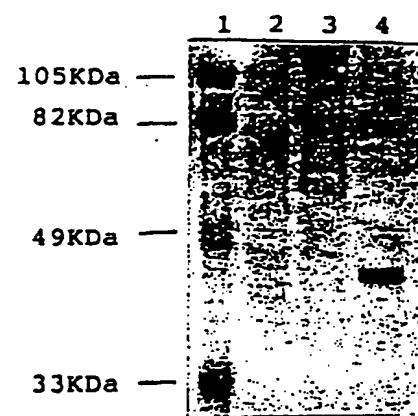
1260	1270	1280	1290	1300
GGCCTCTCAACCAAAAGGTGTCCTCTTTAAGGGCTCGGGTACTGGCAG				
P	L	N	Q	K
V	F	L	F	K
G	S	G	S	Y
Y	W	Q		
1310	1320	1330	1340	1350
TGGGACGAGCTAGCCCGAACTGACTTCAGCAGCTACCCCAAACCAATCAA				
W	D	E	L	A
R	T	D	F	S
S	S	Y	P	K
P	I	K		
1360	1370	1380	1390	1400
GGGTTTGTACGGGAGTGCCAAACCAGCCCTCGGCTGCTATGAGTTGGC				
G	L	F	T	G
V	P	N	Q	V
P	S	A	A	M
S	W		S	Q
1410	1420	1430	1440	1450
AAGATGGCCGAGTCTACTTCCTCAAGGGCAAAGCTACTGGGGCTCAAC				
D	G	R	V	Y
F	K	G	K	V
Y	W	R	L	N
1460	1470	1480	1490	1500
CAGCAGCTTCGAGTAGAGAAAGGCTATCCCAGAAATATTCACAACTG				
Q	Q	L	R	V
R	E	K	G	Y
V	K	P	R	P
E	G	N	N	H
K	Y	S	H	N
1510	1520	1530	1540	1550
GATGCACTGTCGTCCCCGGACTATAGACACTACCCCATCAGGTGGAAATA				
M	H	C	R	P
R	P	T	I	D
T	T	I	D	T
I	D	T	T	P
D	T	T	P	S
T	T	T	P	G
T	T	T	S	G
T	T	T	G	N
T	T	T	A	T
1560	1570	1580	1590	1600
CCACTCCCTCAGGTACGGGCATAACCTTGGATACCACTCTCTCAGCCACA				
T	P	S	G	T
P	S	G	T	I
S	G	T	I	T
G	I	T	L	D
I	L	D	T	T
L	D	T	T	L
D	T	T	L	S
T	T	L	S	A
T	S	A	T	T
1610	1620	1630	1640	1650
GAAACCACGTTGAATACTGACTGCTACCCACAGACACAATCTGGACAA				
E	T	T	F	E
T	F	E	Y	*
1660	1670	1680	1690	1700
TTAACCCCTGAGGCTCCACCAACCCACCCCTTCAATTCCCCCCCCAGAACGCC				

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Fig. 4

1710	1720	1730	1740	1750
TAAGGCCTAATAGCTGAATGAAATACCTGTCTGCTCAGTAGAACCTTGCA				
1760	1770	1780	1790	1800
GGTGCTGTAGCAGGCGCAAGACCGTAGATTCAGGCTTTAACACTTCCA				
1810	1820	1830	1840	1850
ACTCCAGCCACCACTTCCCTGTGCATTTCACTCCTGAGAAGTGCTCCCC				
1860	1870	1880	1890	1900
TAACTCAGATCCCCCTAACTTAGATTGGCCCCCAACTCCATTCCCTGTCT				
1910	1920	1930	1940	1950
GTCTTAGACAGCCCTTCCAACGTGTCACTCTCTCTGGAGGTCAATGG				
1960	1970	1980	1990	2000
TGGAGGGAGATGCCTGGGTCTGTTCTCCTACATAAAATGCAAGAAAAC				
2010	2020	2030	2040	2050
AGCATGGCCAGTAAACTGAGCAAGGGCTTGGAAATCCTTGAGAATCACAT				
2060	2070	2080	2090	2100
TTATGTGCTTATGATTACGGGCAAGCTAACCTAACCTTGTGAATCTCAGA				
2110	2120	2130	2140	2150
TTCCCCATTGCAACATTAGGTAAAGACCCAGTACTGCAGGATTGTGACAC				
2160	2170	2180	2190	2200
TAAATGAAATACTGTATGTGAAGTGCCTGGCACAGTGTCTGGTACATTG				
2210	2220	2230	2240	2250
TGTTTAATAAAAGCTAACCTCCATGTTACATAAGAGAGGACTGAAAAAA				
2260	2270			
AAAAAAAAAAAAAA				

Fig. 5



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Fig. 6 (Cont.)

10	20	30	40	50
GTCCCCCTGCCTAGCCCTGTTCCCTCCAAGTTCCCAGAAGTCTCAGGTAGA				
60	70	80	90	100
GGGCTCAGGCAGCTCTGGAACTCTTGCTGCTGGGACCATGGACTGGCA				
MetAspTrpGln				
110	120	130	140	150
GCACCTGTCGGCTGGCCTTCTTACTTCCTGTGACAGTCTCAGGCCGGGCTC				
GlnLeuTrpLeuAlaPheLeuLeuProValThrValSerGlyArgAlaLeu				
160	170	180	190	200
TGGGGCCTGCAGAGAAGGAGGCGGTGGTGGATTACCTGTTGCAGTATGGG				
GlyProAlaGluLysGluAlaValValAspTyrLeuLeuGlnTyrGly				
210	220	230	240	250
TATCTACAGAAACCTCTGGAAGGAGCTGATGACTTCAGGCTAGAAGATAT				
TyrLeuGlnLysProLeuGluGlyAlaAspAspPheArgLeuGluAspIle				
260	270	280	290	300
CACAGAGGCTTAAGAACTTCCAGGAAGCATCTGAACTCGCCTGTTCCG				
ThrGluAlaLeuArgThrPheGlnGluAlaSerGluLeuProValSerGly				
310	320	330	340	350
GTCAGATGGATGATGCCACAAGGGCCCGTATGAAGCAGCCCCGTTGTGGC				
GlnMetAspAspAlaThrArgAlaArgMetLysGlnProArgCysGly				
360	370	380	390	400
CTGGAGGATCCCTTCAACCAGAAGACTCTGAAATACCTGCTCTGGGCCA				
LeuGluAspProPheAsnGlnLysThrLeuLysTyrLeuLeuLeuGlyHis				
410	420	430	440	450
CTGGAGAAAGAAGCACTTGACATTCCGCATCTGAAACGTGCCCTCCACCC				
TrpArgLysLysHisLeuThrPheArgIleLeuAsnValProSerThrLeu				

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Fig. 6 (Cont.)

460	470	480	490	500
TCTCACCCCTCCAGAGTCCGAGCAGGCCCTGCATCAAGCCTTAAGTATTGG				
SerProSerArgValArgAlaAlaLeuHisGlnAlaPheLysTyrTrp				
510	520	530	540	550
AGCAATGTAGCCCCCTGACCTTCGGGAGGTGAAAGCTGGTTGGCTGA				
SerAsnValAlaProLeuThrPheArgGluValLysAlaGlyTrpAlaAsp				
560	570	580	590	600
TATCCGCCTCTCGTTCCATGGCCGCCAAAGCCCATACTGCTCCAACAGCT				
IleArgLeuSerPheHisGlyArgGlnSerProTyrCysSerAsnSerPhe				
610	620	630	640	650
TTGATGGCCTGGGAAGGTCTGGCCCATGCTGACGTCCCAGAGCTGGC				
AspGlyProGlyLysValLeuAlaHisAlaAspValProGluLeuGly				
660	670	680	690	700
AGTGTACACTTCGATAACGATGAATTCTGGACCGAGGGCACCTACCAGGG				
SerValHisPheAspAsnAspGluPheTrpThrGluGlyThrTyrGlnGly				
710	720	730	740	750
AGTGAACCTACCGCATCATGGCGCCCATGAGGTGGGCCACGCCCTGGGAC				
ValAsnLeuArgIleIleAlaAlaHisGluValGlyHisAlaLeuGlyLeu				
760	770	780	790	800
TTGGGCATTCCCGATATACCCAGGCACCTCATGGCCCTGTTACGCTGGC				
GlyHisSerArgTyrThrGlnAlaLeuMetAlaProValTyrAlaGly				
810	820	830	840	850
TACCAGCCCTACTTCAGGCTGCATCGGATGATGTGGCAGGGATCCAGGC				
TyrGlnProTyrPheArgLeuHisProAspAspValAlaGlyIleGlnAla				

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Fig. 6 (cont.)

860	870	880	890	900
GCTCTATGGCAAGAGGAGGCCGGAGCCAGAAGATGAGGAGGAAGAGGTGG				
LeuTyrGlyLysArgArgProGluProGluAspGluGluGluGluValGlu				
910	920	930	940	950
AGATGCACACTGTGTCAACAGTGACCACAAACCCAGTCCCATGCCAAC				
MetHisThrValSerThrValThrThrLysProSerProMetProAsn				
960	970	980	990	1000
CCCTGCAGCAGTGAAGTGGATGCCATGATGCTAGGCCCTGGGGAAAGAC				
ProCysSerSerGluValAspAlaMetMetLeuGlyProArgGlyLysThr				
1010	1020	1030	1040	1050
CTATGCTTCAAGGGTGAATATGTGTGGACTGTAAACAGATTAGGGCCAG				
TyrAlaPheLysGlyAspTyrValTrpThrValThrAspSerGlyProGly				
1060	1070	1080	1090	1100
GGCCCTTGTCCGAGTGTCTGCCCTTGGGAGGGCTTCTGGAAACCTG				
ProLeuPheArgValSerAlaLeuTrpGluGlyLeuProGlyAsnLeu				
1110	1120	1130	1140	1150
GATGCTGCTGTCTACTCTCCCCGGACACAGCGGACTCATTCTTCAAGGG				
AspAlaAlaValTyrSerProArgThrGlnArgThrHisPhePheLysGly				
1160	1170	1180	1190	1200
AAACAAGGTGTGGCGGTATGTGGATTCAAGTTGTCTCTGGCTTCCCA				
AsnLysValTrpArgTyrValAspPheLysLeuSerProGlyPheProMet				
1210	1220	1230	1240	1250
TGAAACTCAACAGAGTGGAACCCAACCTAGATGCAGCTCTATTGGCCT				
LysLeuAsnArgValGluProAsnLeuAspAlaAlaLeuTyrTrpPro				

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Fig. 6 (Cont.)

1260	1270	1280	1290	1300
GTTAATCAGAACGGTGTCCCTTTAAGGGCTCAGGATACTGGCAATGGGA				
ValAsnGlnLysValPheLeuPheLysGlySerGlyTyrTrpGlnTrpAsp				
1310	1320	1330	1340	1350
TGAACIGACCAGAACTGACCTCAGTCGCTACCCCAAACCAATCAAGGAAC				
GluLeuThrArgThrAspLeuSerArgTyrProLysProIleLysGluLeu				
1360	1370	1380	1390	1400
TTTCACTGGAGTGCCAGACCAACCCTCAGCAGCTATGAGCTGGCAAGAT				
PheThrGlyValProAspGlnProSerAlaAlaMetSerTrpGlnAsp				
1410	1420	1430	1440	1450
GGCCAAGTCTACTTCTCAAGGGCAAAGAGTACTGGCCGCTTAACCAGCA				
GlyGlnValTyrPhePheLysGlyLysGluTyrTrpArgLeuAsnGlnGln				
1460	1470	1480	1490	1500
ACTTCGAGTGGCAAAGGGCTATCCCAGAAATACGACACACTGGATGCACT				
LeuArgValAlaLysGlyTyrProArgAsnThrThrHisTrpMetHisCys				
1510	1520	1530	1540	1550
GTAGTCCTCGGACTCCAGACACTAACTCATTAACTGGGGATGTGACCACT				
SerProArgThrProAspThrAsnSerLeuThrGlyAspValThrThr				
1560	1570	1580	1590	1600
CCTGCAACCGTGGAAATCAGTCTGGATGTCCCTCTGCCACAGACGCTGC				
ProAlaThrValGluSerValLeuAspValProSerAlaThrAspAlaAla				
1610	1620	1630	1640	1650
CTCCCTCTCATCCTCAGCTAACATGTCACCTTGCTAGGGGCCTGAGAACTAG				
SerLeuSerSerAlaAsnValThrLeuLeuGlyAla***				
1660	1670	1680	1690	1700
TCAGTGTCTGCTCCTTAGGGTGTGCAAGATGGCACTTGACCTAGTGCCT				

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Fig. 6

1710 1720 1730 1740 1750

CTAGATACTCCAATTCTGGATGCCACATTCCAGTGTTCCTAGAAAGTGAC

1760 1770 1780 1790 1800

TGCTTAATTCTGAGTCATTCCCCAGTCCTTCTTGTCAATGGC

1810 1820 1830 1840 1850

TGTTTCAAGTGTGACATCTATTTCTGGTGGAGGGAAATTGTGATCAGG

1860 1870 1880 1890 1900

ACCCCCCCCCCCCCCAGGGTCTCTACATAGCACTGGCTATGGTTATCG

1910 1920 1930 1940 1950

GCTATCCTGAAACTGIGTAGTTATGTAGACTAGGCTAACTTGAACTCACA

1960 1970 1980 1990 2000

GAAACCAACCTGCCTCTGCCTCTGTCCTGAGTGCTGGATTAAAAACGTG

2010 2020 2030 2040 2050

TGCTACCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 97/01433

A. CLASSIFICATION OF SUBJECT MATTER	
IPC 6	C12N15/15 C12N9/64 C12N1/21 C12N5/10 A61K38/48
	C07K16/40 C12Q1/37 A61K31/705 A61K39/395 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 97 19178 A (UNIV KONSTANZ ;KRAWINKEL ULRICH (DE); MAUCH SIMON (DE); SEDLACEK R) 29 May 1997 see the whole document ---	1-23
P, X	COSSINS J ET AL: "IDENTIFICATION OF MMP-18, A PUTATIVE NOVEL HUMAN MATRIX METALLOPROTEINASE" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 228, no. 2, 12 November 1996, pages 494-498, XP002036483 see the whole document ---	1-23 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *D* document referring to an oral disclosure, use, exhibition or other means
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- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

1

Date of the actual completion of the international search

17 September 1997

Date of mailing of the international search report

01.10.97

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Van der Schaal, C

INTERNATIONAL SEARCH REPORT

Intern'l Application No
PCT/JP 97/01433

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>PENDAS A M ET AL: "Identification and characterization of a novel human matrix metalloproteinase with unique structural characteristics, chromosomal location, and tissue distribution." JOURNAL OF BIOLOGICAL CHEMISTRY 272 (7). 1997. 4281-4286. ISSN: 0021-9258, 14 February 1997, XP002040910 see the whole document</p> <p>-----</p> <p>PUENTE X S ET AL: "MOLECULAR CLONING OF A NOVEL MEMBRANE-TYPE MATRIX METALLPROTEINASE FROM A HUMAN BREAST CARCINOMA" CANCER RESEARCH, vol. 56, no. 5, 1 March 1996, pages 944-949, XP000644455</p> <p>-----</p>	1-23
A		

INTERNATIONAL SEARCH REPORT

International application No
PCT/JP 97/01433

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely
Remark: Although claim(s) 20 and 22 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 64(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/JP 97/01433

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9719178 A	29-05-97	DE 19543265 A	05-06-97